Response of soil viral and microbial functional diversity to long-term agricultural management in Jackson, West Tennessee

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Ning Duan

December 2021

Copyright © 2021 by Ning Duan

All rights reserved.

ACKNOWLEDGEMENTS

First of all, I would like to acknowledge my supervisor, Dr. Sean Schaeffer, who is always supportive and provide insightful guidance on resolving research questions.

My sincere thank also go to my committee members, Dr. Mark Radosevich, Dr. Margaret Staton, Dr. Jennifer DeBruyn for their significant questions and guidance. I would particularly like to appreciation to Dr. Jie Zhuang, I want to thank you for your support and for the opportunities I was given to pursue my research career.

In addition, I would thank my grandmother, my parents and all my family members who are always there for me. Finally, I could not have completed this dissertation without the support of my friends, Lidong Li and Xiaolong Liang.

ABSTRACT

Soil microbial communities are a critical component for ecosystem stability and function. Viruses, as an important biotic controller, have the potential to regulate the abundance and diversity of bacterial communities through infection. Soil is known to harbor abundant and diverse viral assemblages but their ecological role and influence on microbial processes has not been fully elucidated. Microbes can be influenced by viruses not only from infection but though biogeochemical feedbacks of the "microbial (bacterium–phage–DOC) loop" or "viral shunt". However, we know relatively little about the microbial community and function under the regulation of viruses in soil and how they respond to agricultural management under climate change. The objectives in this dissertation were (1) to estimate variability of soil viral and bacterial communities under a long-term conventional tillage, cover cropping and inorganic N fertilization management practices, and (2) to access the effect of seasonal change on soil bacteria community diversity and structure, and (3) identify the correlation between viruses and their host, and (4) reveal the response of microbial functional genes (C-degradation and N-cycling genes) on cover cropping and fertilization, and the potential roles of viruses on C-degradation. Soil treatments including two nitrogen rates (0, 67 kg N/ha $^{-1}$), three levels of cover crop (no-cover, hairy vetch and winter wheat), and two tillage managements (conventional tillage and no tillage) from West Tennessee Agriculture Research and Education Center in Jackson were used. The soil bacterial diversity, functional gene and viral diversity was evaluated by 16S rRNA amplicon sequencing, RAPD-PCR, and bulk soil metagenomic sequencing. My findings highlight the importance of microbial on N fertilization and cover cropping in maintaining long-term C pool stability and N concentractions in agricultural soil, and the impact

of viruses on C metabolism through regulating microbial metabolism using auxiliary metabolic genes. This study improves our understanding the ecological roles of soil viruses in influencing soil functions under long-term conservation agricultural management.

TABLE OF CONTENTS

Chapter 1 Introduction....1

1.1 Microbial abundance and diversity....2

1.2 Microbial function in terrestrial system....3

1.3 Virus mediated top-down regulation....5

1.4 Seasonal agricultural soil management....8

1.5 Genomic techniques for exploring soil microbial and viral diversity....11

1.6 Research question, hypothesis, and rationale....13

1.7 Approach....19

1.8 Significance....19

References....21

Appendix 1....30

Chapter 2 Composition of soil viral and bacterial communities after long-term tillage, fertilization, and cover cropping management....31

Abstract....33

2.1 Introduction....34

2.2 Materials and methods....36

2.2.1 Study site and soil sampling....36

2.2.2 Whole soil DNA extraction, amplification, and sequencing....37

2.2.3 Amplicon sequence data processing....38

2.2.4 Bacterial diversity and statistical analysis....39

2.2.5 Viral and bacterial particle extraction and enumeration procedure....40

2.2.6 RAPD-PCR and gel visualization and analysis 41
2.2.7 Soil properties 43
2.2.8 Structural equation modeling 44
2.2.9 Statistical analysis 45
2.3 Results 45
2.3.1 Influences of fertilization, tillage and cover cropping on bacterial diversity 46
2.3.2 Influences of tillage, fertilization and cover cropping on viral community composition 47
2.3.3 Correlation among environmental variables, bacterial and viral communities 47
2.4 Discussion 48
2.4.1 Bacterial community composition after 40 years of conservation management 48
2.4.2 Viral diversity patterns in conservation management systems 49
2.4.3 Association between bacterial and viral community 50
2.5 Conclusions 52
References 53
Appendix 2 64
Chapter 3 Seasonal variation in bacterial community structure under long-term fertilization, tillage, and cover cropping 76
Abstract 78
3.1 Introduction 79
3.2 Material and methods 81
3.2.1 Site description and sample collection 81
3.2.2 Pre-processing and DNA extraction 83

3.2.3 Illumina MiSeq sequencing 83
3.2.4 Sequence data analyses 84
3.2.5 Soil properties 85
3.2.6 Alpha-diversity and Beta-diversity measurement 86
3.2.7 Structural equation modeling 86
3.2.8 Statistical analysis 87
3.3 Results 88
3.2.1 Contribution of bacterial phyla and bacterial species involved in nitrogen cycling 89
3.3.2 Seasonal Variations of soil bacteria diversity under long-term managements 90
3.3.3 Comparisons of bacterial community structure across long-term tillage, fertilization, and cover cropping 90
3.3.4 Changes of bacterial genus involve in N fixation, nitrification, and denitrification across long-term management and seasonal environmental change 91
3.4 Discussion 93
3.4.1 Bacterial diversity and communities structure influenced by the agricultural management across the four seasons. 93
3.4.2 Bacterial taxa involving in N-cycling under tillage, fertilization, and cover cropping across seasons. 95
3.5 Conclusions 97
References 98
Appendix 3 111

Chapter 4 Identification of novel viruses and its microbial host from soils with long-term fertilization and cover cropping management 120
Abstract 122
4.1 Introduction 123
4.2 Material and methods 127
4.2.1 Site description and sample collection 127
4.2.2 DNA extraction, library construction, and metagenomic sequencing 127
4.2.3 Reads pre-processing and assembly 129
4.2.4 Viral population recovery reads mapping, and taxonomy identification 129
4.2.5 Metagenome-assembled genome (MAG) construction 131
4.2.6 Building phylogenetic tree for the host genome 132
4.2.7 Virus–host linkage based on CRISPR arrays and BLAST 132
4.2.8 Statistical analysis 133
4.3 Results 134
4.3.1 Dataset overview and agricultural soil viral population (vOTU) recovery 134
4.3.2 Gene-sharing network for viral taxonomy assignment 135
4.3.3 Distribution and diversity of vOTUs across treatments 136
4.3.4 The linkage between viruses and bacterial host 137
4.3.5 The linkage between viruses and archaeal host 139
4.4 Discussion 140
4.4.1 The limitations of using metagenomes to discover soil viromes 140
4.4.2 Taxonomic classification of viral OTUs 142
4.4.3 Inorganic N fertilization influence the viral community diversity and structure 142

4.4.4 The roles of viruses on influencing C and N cycling through the link with functional host.....144
4.5 Conclusions 145
References 146
Appendix 4 193
Chapter 5 The influence of long-term cover cropping and inorganic N fertilization on microbial carbon, nitrogen cycling genes and viral auxiliary metabolic genes.....200
Abstract.....201
5.1 Introduction 203
5.2 Material and methods 206
5.2.1 Site description and sample collection 206
5.2.2 DNA extraction, library construction, and metagenomic sequencing.....206
5.2.3 Reads pre-processing and assembly, and functional gene annotation.....207
5.2.4 Identification of viral auxiliary metabolic genes (AMG) and phylogenetic analyses 208
5.2.5 Statistical analysis.....209
5.3 Results 210
5.3.1 Effects of long-term fertilization and cover cropping on variation on abundances of functional genes related to carbon degradation 210
5.3.2 Effects of long-term fertilization and cover cropping on variation on functional genes involved in N-cycling 212
5.3.3 Viral genes related to carbohydrates metabolism.....213
5.4 Discussion.....214

5.4.1 The influence of management on gene for C decomposition 215
5.4.2 The influence of management on N-cycling gene 216
5.4.3 The function of viral AMGs in microbes and its role in C metabolism 217
5.5 Conclusions 220
References 222
Appendix 5 234
Chapter 6 Conclusions 242
VITA 247

LIST OF TABLES

Table 2.1 Standardized total effects of structural equation model. 69

Table 2.2 The three-way PERMANOVA analysis of bacteria community in RCBD with five significant indicators (Fertilization, cover cropping, tillage, LAP, EOC).....73

Table 2.3 The three-way PERMANOVA analysis of viral community in RCBD with cover crops based upon results of RAPD-PCR.....74

Table 2.4 The coding of sample ID 75

Table 3.1 Results of three-way ANOVA testing based on RCBD model of bacterial alpha diversity. 117

Table 3.2 PERMANOVA analysis for each season 119

Table 4.1 Sequencing information of each soil sample.....193

LIST OF FIGURES

Figure 1.1 Conceptual frame 30

Figure 2.1 The relative abundance of bacteria at phylum level.....64

Figure 2.2 Alpha diversity of bacteria communities across fertilization, tillage, and cover cropping. 65

Figure 2.3 Canonical analysis of principal coordinates (CAP) of bacterial communties and environmental factors 66

Figure 2.4 Canonical analysis of principal coordinates (CAP) of viral communities across treatments.....67

Figure 2.5 Structural equation modeling for relationship among viral, bacteria abundances, and environmental factors. 68

Figure 2.6 Field map.....70

Figure 2.7 The relative abundance of dominant bacteria phyla (> 2% of total relative abundance) across fertilization and tillage management. 71

Figure 2.8 Alpha diversity of bacteria communities 72

Figure 3.1 The contribution (%) of bacteria phyla to dissmilatory of pairwise comparsion among cover cropping, tillage, and fertilization.....111

Figure 3.2 Boxplot of bacteria richness and evenness across four sampling seasons and tillage management.....112

Figure 3.3 Canonical analysis of principal coordinates analysis (CAP) across four seasons under tillage, N fertilization, and cover cropping.....113

Figure 3.4 Spearson's correlation among bacteria at genus level involved in N-fixation, nitrification, and denitrification. 114
Figure 3.5 Structure equation model for N-fixing, nitrifying, and denitrifying bacteria. 115
Figure 3.6 The relative abundance of dominate bacteria phyla collected in May, June, October, and December. 116
Figure 4.1 Gene-sharing network of vOTUs. 194
Figure 4.2 The distribution and diversity of vOTUs recovered in this study across four treatments. 195
Figure 4.3 The link between virus and bacterial host based on the results using CRISPR and BLAST. 196
Figure 4.4 Correlation between abundance of five vOTUs and their host *Verrucomicrobiota AV55*. 197
Figure 4.5 The link between viruses and archaeal host based on the results using BLAST. 198
Figure 4.6 Correlations between vOTUs and their archaeal host *Thermoproteota Nitrososphaeraceae*. 199
Figure 5.1 Gene relative abundances by decomposition of different C substrate. 234
Figure 5.2 Differential abundances analysis on microbial gene for carbohydrate decomposition. 235
Figure 5.3 Redundancy analysis on microbial C decomposition gene. 236
Figure 5.4 Differential abundance analysis on N-cycling gene of microbial communities. 237
Figure 5.5 Pearson's correlation coefficients among soil properties and genes involved in nitrification, denitrification, assimilatory nitrate reduction, assimilatory nitrate reduction. 238

Figure 5.6 Heatmaps for abundances of AMGs related to C decomposition across cover cropping and fertilization treatments. 239
Figure 5.7 Characterization of four auxiliary metabolic genes and microbial genes. 240

Chapter 1 Introduction

Soil is a complex system, up to 10 billions of microbes can be harbored in one gram of soil. Thousands of different microbial species have distinct functions, such as decomposition of organic matter, nutrient cycling, and soil aggregation, etc. (Torsvik & Øvreås, 2002; Griffiths et al., 2004). Microbial diversity plays a vital role in maintaining soil health and sustainable production (Ekschmitt et al., 2005). Ecological function becomes more resilient in a more diverse system, even after disturbance, because there is greater probability that microbes with similar function are present that can replace the ecological function position from specific species to keep system continue working (Botton et al., 2006). However, most of microbes and their functions that have not been explored in most ecosystems.

There are two controls that significantly influence microbial diversity. One control is their "predator" including protists and viruses. Viruses are extremely small particles (~20-250 nm) that can control microbial abundance and community composition through infection, which is "top-down" regulation (Williamson et al., 2017b). Up to now, the progress of studying viral ecology has been slow and received has received relatively little attention. To obtain more complete knowledge of microbial functional dynamics, the interaction between soil viruses and microbes cannot be ignored (Zablocki et al., 2016). The other control is substrate availability, which includes both nutrient and energy sources for microbes. This regulation has been studied widely, it has been called "bottom-up" regulation (Wardle et al., 1998). In an agroecosystem, tillage, nitrogen fertilization and cover cropping will result in the variation of substrates; and soil environmental factors, such as temperature, moisture, pH, redox potential, drive microbial

diversity change (Roesch et al., 2007; Schindlbacher et al., 2011; Zhalnina et al., 2015b). There is also the potential for viral inactivation by enviromental variables such as soil moisture, temperature, nutrients availability, pH, aerobicity, salinity, soil type, which will affect the interactions between viruses and microbes in soil (Ashelford et al., 2000; Kimura et al., 2008b). All of these can influence microbial diversity directly or indirectly through affecting two important regulations mentioned above.

Water availablity and temperature strongly influence viral abundance, inactivation and survival rate in soil (Yeager & O'Brien, 1979a; Hurst et al., 1980a; Zablocki et al., 2016; Williamson et al., 2017b). However, little is known about the variation of microbial community and changes in viral diversity under the effect of seasonal soil agriculture management practices. The goal of my dissertation is to investigate microbial and viral diversity under the influence of tillage, nitrogen fertilization and different cover cropping, and to estimate if the variations in bacterial and viral communities across seasons that vary with temperature and moisture. My objectives are to examine variations of microbial and viral communities and identify C-degradation related functional genes using amplicon sequencing and bulk soil metagenomic sequencing. My research will improve the understanding the ecological role of soil microbes and viruses

1.1 Microbial abundance and diversity

So far, there are still a large number of unknown species with less than 1% of microbes known through cultivation and characterizing (Torsvik & Øvreås, 2002). In soil ecosystems, it has been estimated that more than 10^6 individual species-level operation taxonomic units (OTU) including

bacteria, archaea and fungi can be found in a single soil (Schimel & Schaeffer, 2012). Microbial diversity can be used to determine taxonomic diversity is to study the taxonomic difference of microbial species and taxonomic heterogeneity (species richness and evenness). Ecological traits of microbial species can also be evaluate based on composition and abundance of functional genes (Torsvik & Øvreås, 2002).

1.2 Microbial function in terrestrial system

Microbes provide an important functional and genetic diversity reservoir in soil (Torsvik & Øvreås, 2002). They can have crucial roles in different soil biogeochemical processes, including C, N and other elements cycling in ecosystems (Fierer et al., 2012). In the soil environment, CO_2 fixed by plants through photosynthesis enters the soil. Then the soil microbial community decomposes these organic substrates, releasing CO_2 through respiration, while integrating a portion into their biomass, some of which forms the carbon stored in soil (Williamson et al., 2017b). Microbes are involved in carbon cycling through three ways: 1) uptake of organic carbon and transformation into microbial-derived carbon, 2) microbial extracellular enzyme modification of plant-derived organic matter that is stabilized C in the soil (Liang et al., 2017b), and 3) releasing C into atmosphere through respiration.

Functional gene abundance can be an indicator of the potential functional traits of the microbes in soil. Many enzymes in microbes are specific for decomposing different carbon substrates including lignin, starch, cellulose, hemicellulose, chitin, and pectin. For example, genes *amyA* to *G*, *glucomylase*, *glaA/B* and *agdA* to *G* encode the enzymes to decompose starch (Nakamura et

al., 2006). Gene *cepA* encodes the cellobiose phosphorylase which related cellulose degradation exist in the *Clostridium stercorarium* (Sadie et al., 2011). Neopullulanase hydrolyzed amylose and produced maltose were found in *Bacillus* (Kamasaka et al., 2002). The beta-N-acetylglucosaminidase (NAGase) catabolize chitin to produce release β-1, 4-linked N-acetyl-d-glucosamine (GlcNAc) monosaccharide from chitin, which represent a significant resources of N for microbes (Du et al., 2019). Pectin is a heteropolysaccharide contained in the cell walls of plants and fungi (Schmitz et al., 2019). Pectate lyase produce oligosaccharides with 4-deoxy-α-D-mann-4-enuronosyl groups at their non-reducing ends, which can be secrete by *Pseudomonas fluorescens* and *Erwinia carotovora* (Zucker & Hankin, 1970).

Microbes carrying genes associated with N transformations are important in N-cycling. Genes *amoABC* encode ammonia monooxygenase and the key enzyme for ammonia oxidization and generate hydroxylamine; hydroxylamine can be further oxidized by hydroxylamine oxidoreductase coded by *HAO*. Genes related to nitrification were found in ammonia-oxidizing archaea (AOA) or ammonium-oxidizing bacteria (AOB), including beta- and gammaproteobacteria and *Thaumarchaeota* archaea phyla (Zhou et al., 2015; Zabaloy et al., 2017). Genes *nirB* and *nirK/S* are associated with nitrite reduction and are involved in dissimilatory nitrate reduction and denitrification pathways, respectively (Zhang et al., 2019c; Heo et al., 2020).

1.3 Virus mediated top-down regulation

Microbes involved in biogeochemical cycles and food chains not only need to maintain balance in their own species community, but are also in dynamic equilibrium with their "predator" (Torsvik & Øvreås, 2002). Viruses are non-cellular life forms, who need to infect host cells for survival and reproduction (Koonin et al., 2006). Viruses, as one of the bio-controllers that regulate the biological populations, have gradually shown their vital roles in ecological process via regulating microbial communities in soil to promote nutrient flow (Allen et al., 2010b) (Mühling et al., 2005; Kimura et al., 2008b), and the composition of prokaryote communities (Weinbauer & Rassoulzadegan, 2004a).

There are several lines of evidence showing how viruses have the ability to influence microbial diversity. Firstly, the abundance of viruses is multiple times greater than that of microbes. Approximately 10^{31} viral-like particles (VLPs) are estimated to be contained in the global "virosphere", making it the most abundant biological and genetic reservoir on earth (Zablocki et al., 2016). Viruses, as a bacterial predator, are distributed widely. There are many viruses in both aquifer and terrestrial systems, it has been estimated that total phage abundance in the biosphere is around 10^{30} or more. In soil systems, viruses and virus-like particle content in soils can range from 1.5×10^{8} to 4.1×10^{9} g^{-1}, which is an order of magnitude greater than microbial abundance in soil (Wommack et al., 2000; Adriaenssens et al., 2015; Cobián Güemes et al., 2016). In aquatic systems, there are on the order of 10^{7} viruses per milliliter (Mann, 2005). Such great viral numbers and infection frequencies of bacteria suggest that viruses may influence bacteria diversity (Weinbauer & Rassoulzadegan, 2004b; Mann, 2005). In soil, many microorganisms can

be viral hosts, like bacteria, archaea, fungi, protists, etc. Bacteriophages have received special attention because studies show that they have the most abundance in soil. For now, only around 10^4 bacterial species per gram soil have been investigated which can be infected by bacterial phages. According to Williamson et al. (2017), only 11 bacterial and 2 archaeal phyla have been described in around two thousand research papers, and the hosts only distributed within 3 phyla: Gammaproteobacteria, Firmicutes, and Actinobacteria; highlighting a large gap in our knowledge of viral diversity (Schloss & Handelsman, 2006; Williamson et al., 2017b). The estimated richness of viral genotypes is distinct in different soil types, which can range from 10^3 to 10^6, with over 50% of sequences not affiliated with any known viral sequences in database (Williamson et al., 2017b). It is clear that the soil virome diversity is seriously underestimated, and it also has great ecological functional potential and is a genetic reservoir that needs to be explored.

Secondly, viruses have the ability infect host cells through three strategies: lytic, lysogenic and pseudo lysogenic infection (Kimura et al., 2008b; Ghosh et al., 2009b). In general, up to half of bacterial mortality may be the explained by viral induced lysis (Shibata et al., 1997). Lytic reproduction, which can lead to cell death, occurs more frequently under higher temperature and lower moisture, which induces physiological stress (Williamson et al., 2017b). Lytic reproduction in response to host physiological stress is a common strategy in marine and freshwater environments (Kimura et al., 2008b). Viral DNA, as a separate free molecule, exists in the bacterial cell, and replicates separately from the host bacterial DNA, induces synthesis of proteins, then destroys the host cell releasing the progeny viruses. Compared with lytic the strategy, lysogeny is a more effective strategy when the host abundance is low or inactive. The

viral genes are integrated into the host bacterium's genome, prophages then replicate as the host cell grows and divides (the host will keep living normally in lysogenic cycle). Then, when the infected host is under the physiological stress or other agents, such as mitomycin C (mitC), UV light, antibiotics, and other chemicals, prophage induction can occur and trigger entry into the lytic cycle (Kimura et al., 2008b; Ghosh et al., 2009a). Researchers define primarily lytic viruses, which have short generation time and higher production rates, as r-strategists, and lysogenic viruses, which have relative longer generation times and low reproductive rates, as K-strategists (Suttle, 2005). Pseudolysogenic infection is where viral nucleic acid are unstable and in an inactive state in their host, usually the host is in an starved state, thus preventing phages from entering into a lytic cycle (Kimura et al., 2008b).

Thirdly, the viruses can influence important microbial functional groups and organic nutrient composition, which in turn affect overall microbial diversity. For example, unicellular cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are the major constituents of microbial communities in upper illuminated layers of the oceans, which contribute to CO_2 fixation and O_2 production. Phage infection of these important functional groups would obviously affect carbon cycling in this case (Mann, 2005). When viruses infect microbes and induce lysis, the organic matter in microbial biomass will be released into the environment. Organic substrate composition will change which will then influence the microbial community structure. Viruses are regarded as a main driving force to influence bacterial community to promote the global nutrients flow (Fuhrman, 1999b; Mann, 2005). In marine environments, virus infection is a major reason for bacterial mortality (Fuhrman, 1999b). Bacterial cells are broken down into cellular debris, which consist of dissolved molecules, including monomers, oligomers

and polymers, also colloids (Shibata et al., 1997) and other cell fragments, but most of carbon produced by viral lysis bacteria are dissolved organic carbon. It has been verified experimentally that a large proportion of the lysis products are fed on by the surviving bacteria, which accelerates their own growth (Fuhrman, 1999b). Therefore, most of carbon is recycled in bacterium–virus–dissolved organic C loop (viral shunt). In the soil environment, CO_2 fixed by plants through photosynthesis goes into the soil, the soil microbial community decomposes this organic matter, turning it into either: CO_2 through respiration, integrating it into their biomass, or forming the recalcitrant carbon stored in soil (Williamson et al., 2017b). However, under the impact of viral lysis, microbial biomass carbon increase means that the amount of soluble labile carbon may increase, leaving less C available for long-term storage in stable forms.

1.4 Seasonal agricultural soil management

Soil biodiversity provides a vital contribution to soil stability, productivity, and resilience (Torsvik & Øvreås, 2002; Gliessman & Tittonell, 2015). Soil microorganism are decomposers and nutrient transformers, and promote various biogeochemical cycles (BGC) through redox reactions (Timonen et al., 1996; Trevors & Pollution, 1998; Kibblewhite et al., 2008; Meliani et al., 2012). Cover cropping, nitrogen fertilization, tillage and other soil management have a complicated influence on soil physical and chemical properties, which may alter the composition and functional structure of microorganism communities in soil (Zak et al., 1994; Bending et al., 2002).

Cropping systems in our study included hairy vetch (*Vicia villosa Roth*) and winter wheat

(*Triticum aestivum. L*). Hairy vetch as a winter cover crop which can fix as much as 200 lb/acre of atmospheric nitrogen. Winter wheat (*Triticum aestivum. L*) is the third most common field crop grown in the United States in terms of planted acreage and total production, behind maize and soybean (Djaman et al., 2018). Cover crop residues serve as physical protection from soil erosion but are also a source of additional organic carbon to soil microbes. Plant residues influence the C:N of organic matter that lead to nitrogen mineralization and immobilization, that determine the inorganic nitrogen availability for plants, which in turn alter microbial activity and structure. Vetch, having a lower C:N ratio, would also have more labile residue than that of wheat, resulting in higher decomposition rates that would in turn lead to the greater accumulation of decomposed organic matter (Mbuthia, 2014). Therefore, crop residue and soil organic matter quality have effect on the soil microbial function diversity (Bending et al., 2002).

Inorganic nitrogen fertilization decreases the soil pH leading to soil acidity, which will affect the microbial enzyme activity, nutrient availablity and solubility of metal for microbes (Rousk et al., 2009). Also, fertilization increases plant growth and thus the soil organic material input (plant residues, root exudates, etc). Thus, microbes are senstive to nitrogen input as it will change their biomass and community structure (Geisseler & Scow, 2014; Zhou et al., 2017). Increasing the nitrogen application rate can promote soil microbial abundance (Treseder, 2008; Ramirez et al., 2010c) and diversity (Mbuthia, 2014).

Tillage practice affects soil water content, temperature, aeration and the degree of mixing of crop residues (Kladivko & Research, 2001; Lienhard et al., 2013). In many cases, tillage is associated with soil erosion, decreases in organic matter, soil fertility and water infiltration, broken soil

structure and a reduction of whole soil quality (Simmons & Coleman, 2008). In terms of the microbial community, reduced tillage has been found to lead to a greater abundance of anaerobic microbial species (Linn & Doran, 1984b; Simmons & Coleman, 2008). However, it has been shown that tillage will disrupt the abundance of mycorrhiza, braking the their hyphal network to influence the plant nutrient absorption (Drijber et al., 2000; Simmons & Coleman, 2008; Mbuthia, 2014).

Soil temperature and mositure content are the key environmental factors that influence viruses. Temperture can accelerate the infection process of viruses (Reanney & Marsh, 1973). However, higher enzyme activities, such as proteases, occurs under the higher temperature in microbes, and can inactive or decompose viral structural proteins, causing viruses lose their ability of host infection. (Kimura et al., 2008b). In addition to the influence of temperature on viruses, under different level of soil moisture, microbes adjust their cell osmotic pressure to response the soil moisture which may influence their metabolism and surviving (Xiang et al., 2008). Soil virus abundance significantly correlates with moisture content (Williamson et al., 2005a). The loss of infectivity of virus happened in both drying and moist soil (Yeager & O'Brien, 1979a). The nuclear acid of viruses were damaged in saturated condition, the disassociation happened between nuclear acid and isoelectrically altered capsids in drying soil (Yeager & O'Brien, 1979b; Kimura et al., 2008b).

1.5 Genomic techniques for exploring soil microbial and viral diversity

There are many fingerprint techniques used to describe community diversity, such as phospholipid fatty acid (PLFA) analysis, denaturant gradient gel electrophoresis (DGGE), amplified rDNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism (T-RFLP) and ribosomal intergenic spacer analysis (RISA). The changes of whole community structure changes can be captured by these high-resolution techniques. However, all of them are too general to get insight to the taxonomic information in each ecological sample. Therefore, we employed three well-developed approaches to provide us the genetic information of viral and microbial diversity.

Small subunit ribosomal RNA (16S rRNA) gene sequencing is used for evaluating prokaryotic communities. The 16S rRNA (1542bp) is long enough contain species information and also have special character become phylogenetic markers almost exist in all the bacteria species. It contains a stable region and variable region, the stable sequences is highly conserved and changes very slowly with time, therefore, many primers are designed to target the stable region, and we recognize the different species by the variable region they amplified (Janda & Abbott, 2007). The 16S rRNA gene sequencing is widely used for classifying bacterial taxonomy and analyzing bacterial diversity.

Shotgun metagenomic sequencing can examine the whole genomes of microbial communities in a given sample, and can target both cultured and uncultured microbial species, including bacteria, archaea, protists, fungi and viruses (Handelsman, 2004; Fierer et al., 2012). Whole soil

metagenomic sequencing has been used to reveal new viruses, genomic diversity, function of uncultured bacteria, and metabolic pathways. This technique also helps us get insight into and their ecological potential function. Generally, there mainly have three steps for analyzing metagenomic data, marker gene analysis is used to compare metagenomic reads to a database contained taxonomic information and find reads which have homologs with marker gene, and then quantify the taxonomic diversity of metagenome computationally. Next, binning is to assign each metagenomic sequence to a taxonomic group through comparing to the reference data or shared characteristics, like GC content. Finally, short reads will be assembled to a single contiguous sequences which is used to generate longer sequences computationally (Sharpton, 2014). Moreover, the metagenomic sequences information on ecological function can be explored through identifying reads which including protein coding sequences and comparing the sequences to the known genes, protein, protein families or metabolic pathways. There are two steps for access the metagenome functional annotation: gene prediction and annotation. Gene prediction is to determine which metagenomic containing protein coding sequences, and then gene annotation is accomplished via grouping the predicted metagenomic proteins into protein families (Sharpton, 2014).

Viruses have no universal signature gene (e.g. 16S rRNA gene) which can be used to identify taxonomy, therefore, metaviromic sequencing is currently the only accurate approach to access the total viral diversity in a habitat (Figure 1) (Adriaenssens et al., 2015). With the advances in next-generation-sequencing (NGS) technologies, the fast growing viral sequencing data indicate that around 70% of virome sequencing data have no homologs in online public databases (Zablocki et al., 2016). Since 2005, there are about 14 excellent papers that have been published

on viral ecology in distinct desert sample locations. Most of them use fingerprint techniques and first generation sequencing to evaluate the viral community, such as Random Amplification of Polymorphic DNA-Polymerase chain reaction (RAPD-PCR), and Sanger sequencing (Zablocki et al., 2016). Next-Generation sequencing on Illumina sequencing platform are a rapidly emerging tool to efficiently and accurately estimate the influence of soil viruses on microbial communities and functions (Zablocki et al., 2014; Adriaenssens et al., 2015; Heather & Chain, 2016).

1.6 Research question, hypothesis, and rationale

Q1. Will bacterial and viral communities be influence by the long-term tillage, cover crop, and fertilization treated soil? (CHAPTER 2)

Hypothesis

Inorganic N fertilization, conventional tillage, and cover cropping has a significant effect on bacterial and viral community structure. Greater bacterial diversity will be observed in cover cropped, fertilized and no-tilled soil than other treatments. Viral abundances will have a positive correlation with bacterial abundances.

Rationale

Inorganic N fertilization and cover cropping increase soil organic matter input, providing N and C resources to for bacterial growth and metabolism (Mbuthia et al., 2015a). No tillage soil will reduce the extremes in soil temperature, keep the soil structure steady and improve water holding capacity and soil moisture providing a suitable environment for bacteria growth (Kladivko, 2001). Bacteria diversity should be higher when compared with soil with no fertilization and no cover cropping and tillage. Bacterial community structure will respond to the quantity and quality of organic matter input and environmental factor change result from agricultural management practices. Therefore, all the practices will have a significant influence on bacterial community structure.

Inorganic N fertilization, cover cropping, and conventional tillage influence the amount and types of organic matter input, soil pH, and soil moisture (Vukicevich et al., 2016a; Finney et al., 2017; Zhou et al., 2017). These changes affect the movement of viruses in the soil, and their distribution and reproduction. Therefore, it might also shift viral community structure. Viral abundances are often closely correlated with bacterial abundances. A net positive lytic viral production would be activated when the competitive bacterial species with dominated abundance and activity (‘kill the winner’ mechanism) (Thingstad & Lignell, 1997; Weinbauer & Rassoulzadegan, 2004a). The viral lysate as readily organic matter can be recycled to feed surviving bacteria and promote growth of non-infected and resistant bacterial cells(Middelboe, 2000). Therefore, the bacterial and viral abundances should positively correlate.

Q2. Will bacterial community diversity and structure be influenced by management across seasons? (CHAPTER 3)

Hypothesis

Inorganic N fertilization, cover cropping, and no-tillage soil should have greater bacterial diversity than other treatment in each season. The highest diversity will be observed in the spring and early summer.

Rationale

Application of fertilizer nitrogen increases crop growth, changes soil organic matter content and decreases the soil pH, which is undesirable for the sustainable development of crop production (Rousk et al., 2009). Also, nitrogen fertilizers lead to soil acidity, which can affect microbial growth, nutrient availablity and solubility of metal ions for microbes (Rousk et al., 2009). Relative abundance and diversity of bacteria tend to be positively related to soil pH (Rousk et al., 2010a). Furthermore, inorganic N inputs improve plant growth, and more plant residues and root exudates are added into the soil, which increases soil organic matter and carbon sequestration. Bacterial biomass and community structure also significantly respond to fertilization (Ramirez et al., 2010b; Geisseler & Scow, 2014; Zhou et al., 2017). Disruption of soil structure via tillage practices decrease soil water content, increase aeration and the degree of mixing of crop residues thereby altering microenvironments and impacting bacteria community structure through bottom up environmental controls (Kladivko, 2001; Lienhard et al., 2013).Tillage would have negative

effect on bacteria communites.

Soil microbes are sensitive to soil temperature and moisture content. The main differences in seasons are temperature and precipitation different. Soil moisture can help dissolve nutrients and microbe movement to make nutrients more available (Stres et al., 2008a). Temperature positively correlated with soil microbial activity within a certain range, soil bacteria have higher enzyme activity and metabolic rate at 25~30℃ (Pettersson & Baath, 2003). In spring and early summer, there have a relative higher temperature and precipitation, therefore, we should find higher diversity in bacterial community.

Q3. How does inorganic N fertilization and a vetch cover crop influence viral community? How do viruses relate to microbial host? (CHAPTER 4)

Hypothesis

Inorganic N fertilization and vetch cover should increase the diversity of viral communities and shape viral community structure. Viruses and their host should be positively correlated in abundances. Viruses may influence soil C and N-cycling by influencing functional microbial taxa.

Rationale

Organic matter and variations of soil properties all affect adsorption/desorption and movement of viral particles in soil, and then influence viral community structure. Viral diversity largely depend on genetic material communication with microbial host (Abedon, 2011). N fertilization and vetch cover crops would provide C and N resources for microbial growth. When microbes are limited by nutrient availability, the surviving strategy of bacteria would change (Nicholson et al., 2000). Phages could respond to the bacterial signals and modifying the outcome of their infection from obligately productive to temporarily reductive (Abedon, 2009; Gabiatti et al., 2018). Therefore, viral community structure would be influenced by N fertilization and vetch cover crops. Soil with fertilization and cover crops would contain greater viral diversity than other treatments.

The viral host were identified in *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Nitrospirae*, *Desulfobacterota* in soil environment (Williamson et al., 2017a; Emerson et al., 2018; Trubl et al., 2018a; ter Horst et al., 2020). All these phyla involved in C and N-cycling, therefore, we expected to find the connection between viruses and functional taxa.

Q4. Will bacterial functional genes controlling C-degradation and N-cycling be influenced by long-term tillage, cover crop, and fertilization? How are viruses involved in C-degradation? (CHAPTER 5)

Hypothesis

More chemically labile C degradation genes will be found in cover cropping with fertilized soil than the soil without cover and fertilization. Genes related to recalcitrant C decomposition should have higher abundance in no fertilization and no cover cropping soil than other treatments. Gene abundances involved in nitrification and denitrification will be stimulated when soil with N fertilizer applied and/or vetch covered. Genes for dissimilatory nitrate reduction might increase under no fertilization and no cover crops treatment.

Rationale

In a long term of cover cropping soil with fertilization, greater plant residues input and soil labile organic carbon, will have more labile carbon decomposition related genes than soil without cover cropping and fertilization. When the soil lacks fresh organic matter input, microbes tend to decompose recalcitrant C component to fulfill their energy and N consumption (Neff et al., 2002; Moorhead & Sinsabaugh, 2006; Craine et al., 2007). N fertilizer and vetch cover crops would provide greater N resources for microbes, and the nitrification process would be enhanced, and extra N might be lost through denitrification process (Skiba & Smith, 1993; Abbasi & Adams, 2000; Ouyang et al., 2018; Wang et al., 2018a). N-deficit plot might increase the N retention pathways for microbial growth (Pandey et al., 2019). Moreover, the roles of viruses involved in N and C-cycling on microbial host in soil has been verified via AMGs (Thompson et al., 2011; Enav et al., 2014; Huang et al., 2021). Most of the AMG genes identified in soil participate carbohydrate, amino acid, energy metabolism, etc. (Emerson et al., 2018; Trubl et al., 2018a;

Graham et al., 2019; ter Horst et al., 2020). Therefore, we assumed that AMGs can be found in soil that involve in C-degradation.

1.7 Approach

The field experiment was conducted for one year, sampling times were 4 per year between May, 2018 to December 2019. The specific sampling times happened in winter (roughly corresponding to the peak in the winter cover crop), spring (shortly after burn-down of the cover crop and just before planting), mid-summer (at the peak of the crop growing season), and fall (following crop harvest). Samples collected in 2018 were processed using 16S rRNA amplicon sequencing and RAPD-PCR. The same amplicon sequencing was performed on samples collected in 2019 on an Illumina MiSeq platform. Samples were selected based upon the previous results and using bulk soil metagenomic sequencing on Illumina NovaSeq platform to examine viral community, host community, and microbial functional gene related to C degradation and N-cycling, and auxiliary metabolic genes.

1.8 Significance

Keeping nutrients storage and balance is critical to maintaining soil fertility and promoting food, fiber production. Compared with substrate control, less is known about the influence of soil viruses on microbial community function, and even less is known about microbial diversity and ecosystem function under the top-down regulations by viruses in soil systems. To fully understand this process, it is necessary to figure out the correlation between diversity of viral

communities and bacterial and environment factors which could induced these changes. This will reveal microbial function at the genetic level and provide the genetic evidence for microbial functional changes, which will help to keeping soil health and develop a sustainable agriculture system.

References

Adriaenssens, E. M., Van Zyl, L., De Maayer, P., Rubagotti, E., Rybicki, E., Tuffin, M., Cowan, D. A. 2015. Metagenomic analysis of the viral community in Namib Desert hypoliths. Environ Microbiol*,* 17(2), 480-495.

Allen, B., Willner, D., Oechel, W. C., Lipson, D. J. E. m. 2010. Top-down control of microbial activity and biomass in an Arctic soil ecosystem. 12(3), 642-648.

Ashelford, K. E., Norris, S. J., Fry, J. C., Bailey, M. J., Day, M. J. 2000. Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere. Appl Environ Microbiol*,* 66(10), 4193-4199.

Bending, G. D., Turner, M. K., Jones, J. E. J. S. B., Biochemistry. 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. 34(8), 1073-1082.

Botton, S., van Heusden, M., Parsons, J. R., Smidt, H., van Straalen, N. 2006. Resilience of microbial systems towards disturbances. Crit Rev Microbiol*,* 32(2), 101-112.

Cobián Güemes, A. G., Youle, M., Cantú, V. A., Felts, B., Nulton, J., Rohwer, F. J. A. R. o. V. 2016. Viruses as winners in the game of life. 3, 197-214.

Djaman, K., O'Neill, M., Owen, C., Smeal, D., West, M., Begay, D., Allen, S., Koudahe, K., Irmak, S., Lombard, K. J. A. 2018. Long-Term Winter Wheat (Triticum aestivum L.) Seasonal Irrigation Amount, Evapotranspiration, Yield, and Water Productivity under Semiarid Climate. 8(6), 96.

Drijber, R. A., Doran, J. W., Parkhurst, A. M., Lyon, D. J. S. B., Biochemistry. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. 32(10), 1419-1430.

Du, C., Jiang, S., Jiang, S., Zhou, Y., Zhang, G. 2019. A Bacillus pumilus originated β-N-acetylglucosaminidase for chitin combinatory hydrolysis and exploration of its thermostable mechanism. International journal of biological macromolecules, 132, 1282-1289.

Ekschmitt, K., Liu, M., Vetter, S., Fox, O., Wolters, V. J. G. 2005. Strategies used by soil biota to overcome soil organic matter stability—why is dead organic matter left over in the soil? Geoderma, 128(1-2), 167-176.

Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., Owens, S., Gilbert, J. A., Wall, D. H., Caporaso, J. G. J. P. o. t. N. A. o. S. 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences, 109(52), 21390-21395.

Fuhrman, J. A. J. N. 1999. Marine viruses and their biogeochemical and ecological effects. 399(6736), 541.

Geisseler, D., Scow, K. M. 2014. Long-term effects of mineral fertilizers on soil microorganisms–A review. Soil Biology and Biochemistry, 75, 54-63.

Ghosh, D., Roy, K., Williamson, K. E., Srinivasiah, S., Wommack, K. E., Radosevich, M. J. A., microbiology, e. 2009a. Acyl-homoserine lactones can induce virus production in lysogenic bacteria: an alternative paradigm for prophage induction. 75(22), 7142-7152.

Ghosh, D., Roy, K., Williamson, K. E., Srinivasiah, S., Wommack, K. E., Radosevich, M. J. A., microbiology, e. 2009b. Acyl-homoserine lactones can induce virus production in lysogenic bacteria: an alternative paradigm for prophage induction. Applied and environmental microbiology, 75(22), 7142-7152.

Gliessman, S., Tittonell, P. (2015). Agroecology for food security and nutrition. In: Taylor & Francis.

Griffiths, R. I., Manefield, M., Ostle, N., McNamara, N., O'Donnell, A. G., Bailey, M. J., Whiteley, A. S. 2004. 13CO2 pulse labelling of plants in tandem with stable isotope probing: methodological considerations for examining microbial function in the rhizosphere. J Microbiol Methods, 58(1), 119-129.

Handelsman, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev, 68(4), 669-685.

Heather, J. M., Chain, B. 2016. The sequence of sequencers: The history of sequencing DNA. Genomics, 107(1), 1-8.

Heo, H., Kwon, M., Song, B., Yoon, S. 2020. Involvement of NO3− in ecophysiological regulation of dissimilatory nitrate/nitrite reduction to ammonium (DNRA) is implied by physiological characterization of soil DNRA bacteria isolated via a colorimetric screening method. Applied and environmental microbiology, 86(17), e01054-01020.

Hurst, C. J., Gerba, C. P., Cech, I. 1980. Effects of environmental variables and soil characteristics on virus survival in soil. Appl Environ Microbiol, 40(6), 1067-1079.

Janda, J. M., Abbott, S. L. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol, 45(9), 2761-2764.

Kamasaka, H., Sugimoto, K., Takata, H., Nishimura, T., Kuriki, T. 2002. Bacillus stearothermophilus neopullulanase selective hydrolysis of amylose to maltose in the presence of amylopectin. Applied and environmental microbiology, 68(4), 1658-1664.

Kibblewhite, M., Ritz, K., Swift, M. J. P. T. o. t. R. S. o. L. B. B. S. 2008. Soil health in agricultural systems. 363(1492), 685-701.

Kimura, M., Jia, Z.-J., Nakayama, N., Asakawa, S. J. S. S., Nutrition, P. 2008. Ecology of viruses in soils: past, present and future perspectives. 54(1), 1-32.

Kladivko, E. J. J. S., Research, T. 2001. Tillage systems and soil ecology. 61(1-2), 61-76.

Koonin, E. V., Senkevich, T. G., Dolja, V. V. 2006. The ancient Virus World and evolution of cells. Biol Direct*,* 1, 29.

Liang, C., Schimel, J. P., Jastrow, J. D. J. N. M. 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology*,* 2(8), 17105.

Lienhard, P., Tivet, F., Chabanne, A., Dequiedt, S., Lelièvre, M., Sayphoummie, S., Leudphanane, B., Prévost-Bouré, N. C., Séguy, L., Maron, P.-A. J. A. f. s. d. 2013. No-till and cover crops shift soil microbial abundance and diversity in Laos tropical grasslands. 33(2), 375-384.

Linn, D., Doran, J. J. S. S. S. o. A. J. 1984. Aerobic and Anaerobic Microbial Populations in No-till and Plowed Soils 1. 48(4), 794-799.

Mann, N. H. J. P. b. 2005. The third age of phage. 3(5), e182.

Mbuthia, L. W. 2014. Long-Term Impacts of Tillage, Cover Crops, and Nitrogen Rates on Microbial Community Dynamics and Soil Quality Parameters under Continuous Cotton Production in West Tennessee.

Meliani, A., Bensoltane, A., Mederbel, K. J. A. J. o. P. N., Technology, F. 2012. Microbial diversity and abundance in soil: related to plant and soil type. 2(1), 10-18.

Mühling, M., Fuller, N. J., Millard, A., Somerfield, P. J., Marie, D., Wilson, W. H., Scanlan, D. J., Post, A. F., Joint, I., Mann, N. H. J. E. m. 2005. Genetic diversity of marine Synechococcus and co-occurring cyanophage communities: evidence for viral control of phytoplankton. 7(4), 499-508.

Nakamura, T., Maeda, Y., Tanoue, N., Makita, T., Kato, M., Kobayashi, T. 2006. Expression profile of amylolytic genes in Aspergillus nidulans. Biosci Biotechnol Biochem, 70(10), 2363-2370.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., Fierer, N. J. E. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. 91(12), 3463-3470.

Reanney, D., Marsh, S. 1973. The ecology of viruses attacking Bacillus stearothermophilus in soil. Soil Biology Biochemistry, 5(4), 399-408.

Roesch, L. F., Fulthorpe, R. R., Riva, A., Casella, G., Hadwin, A. K., Kent, A. D., Daroub, S. H., Camargo, F. A., Farmerie, W. G., Triplett, E. W. J. T. I. j. 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. 1(4), 283.

Rousk, J., Brookes, P. C., Baath, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol, 75(6), 1589-1596.

Sadie, C. J., Rose, S. H., den Haan, R., van Zyl, W. H. 2011. Co-expression of a cellobiose phosphorylase and lactose permease enables intracellular cellobiose utilisation by Saccharomyces cerevisiae. Appl Microbiol Biotechnol, 90(4), 1373-1380.

Schimel, J., Schaeffer, S. M. J. F. i. m. 2012. Microbial control over carbon cycling in soil. 3, 348.

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S. J. S. B., Biochemistry. 2011. Experimental warming effects on the microbial community of a temperate mountain forest soil. 43(7), 1417-1425.

Schloss, P. D., Handelsman, J. 2006. Toward a census of bacteria in soil. PLoS Comput Biol, 2(7), e92.

Schmitz, K., Protzko, R., Zhang, L., Benz, J. P. 2019. Spotlight on fungal pectin utilization—from phytopathogenicity to molecular recognition and industrial applications. Applied Microbiology and Biotechnology, 103(6), 2507-2524.

Sharpton, T. J. 2014. An introduction to the analysis of shotgun metagenomic data. Front Plant Sci, 5, 209.

Shibata, A., Kogure, K., Koike, I., Ohwada, K. J. M. E. P. S. 1997. Formation of submicron colloidal particles from marine bacteria by viral infection. 155, 303-307.

Simmons, B. L., Coleman, D. C. J. A. s. e. 2008. Microbial community response to transition from conventional to conservation tillage in cotton fields. 40(3), 518-528.

Srinivasiah, S., Lovett, J., Polson, S., Bhavsar, J., Ghosh, D., Roy, K., Fuhrmann, J. J., Radosevich, M., Wommack, K. E. 2013. Direct assessment of viral diversity in soils by random PCR amplification of polymorphic DNA. Appl Environ Microbiol, 79(18), 5450-5457.

Suttle, C. A. 2005. Viruses in the sea. Nature, 437(7057), 356-361.

Tan, J., Reanney, D. 1976. Interactions between bacteriophages and bacteria in soil. Soil Biology Biochemistry, 8(2), 145-150.

Timonen, S., Finlay, R. D., Olsson, S., Söderström, B. J. F. M. E. 1996. Dynamics of phosphorus translocation in intact ectomycorrhizal systems: non-destructive monitoring using a β-scanner. 19(3), 171-180.

Torsvik, V., Øvreås, L. 2002. Microbial diversity and function in soil: from genes to ecosystems. Current opinion in microbiology, 5(3), 240-245.

Treseder, K. K. J. E. l. 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. 11(10), 1111-1120.

Trevors, J. J. W., Air,, Pollution, S. 1998. Bacterial biodiversity in soil with an emphasis on chemically-contaminated soils. 101(1-4), 45-67.

Wardle, D. A., Verhoef, H. A., Clarholm, M. J. G. C. B. 1998. Trophic relationships in the soil microfood-web: Predicting the responses to a changing global environment. 4(7), 713-727.

Weinbauer, M. G., Rassoulzadegan, F. 2004a. Are viruses driving microbial diversification and diversity? Environ Microbiol*,* 6(1), 1-11.

Weinbauer, M. G., Rassoulzadegan, F. J. E. m. 2004b. Are viruses driving microbial diversification and diversity? *,* 6(1), 1-11.

Williamson, K. E., Fuhrmann, J. J., Wommack, K. E., Radosevich, M. J. A. r. o. v. 2017. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. 4, 201-219.

Williamson, K. E., Radosevich, M., Wommack, K. E. 2005. Abundance and diversity of viruses in six Delaware soils. Appl Environ Microbiol*,* 71(6), 3119-3125.

Wommack, K. E., Colwell, R. R. J. M., reviews, m. b. 2000. Virioplankton: viruses in aquatic ecosystems. 64(1), 69-114.

Xiang, S.-R., Doyle, A., Holden, P. A., Schimel, J. P. J. S. B., Biochemistry. 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biology and Biochemistry*,* 40(9), 2281-2289.

Yeager, J. G., O'Brien, R. T. 1979a. Enterovirus inactivation in soil. Appl Environ Microbiol*,* 38(4), 694-701.

Yeager, J. G., O'Brien, R. T. 1979b. Structural changes associated with poliovirus inactivation in soil. Appl Environ Microbiol, 38(4), 702-709.

Zabaloy, M. C., Allegrini, M., Tebbe, D. A., Schuster, K., Gomez, E. d. V. 2017. Nitrifying bacteria and archaea withstanding glyphosate in fertilized soil microcosms. Applied Soil Ecology, 117, 88-95.

Zablocki, O., Adriaenssens, E. M., Cowan, D. 2016. Diversity and Ecology of Viruses in Hyperarid Desert Soils. Appl Environ Microbiol, 82(3), 770-777.

Zablocki, O., van Zyl, L., Adriaenssens, E. M., Rubagotti, E., Tuffin, M., Cary, S. C., Cowan, D. 2014. High-level diversity of tailed phages, eukaryote-associated viruses, and virophage-like elements in the metaviromes of antarctic soils. Appl Environ Microbiol, 80(22), 6888-6897.

Zak, J. C., Willig, M. R., Moorhead, D. L., Wildman, H. G. J. S. B., Biochemistry. 1994. Functional diversity of microbial communities: a quantitative approach. 26(9), 1101-1108.

Zhalnina, K., Dias, R., de Quadros, P. D., Davis-Richardson, A., Camargo, F. A., Clark, I. M., McGrath, S. P., Hirsch, P. R., Triplett, E. W. J. M. e. 2015. Soil pH determines microbial diversity and composition in the park grass experiment. 69(2), 395-406.

Zhang, L., Jiang, M., Ding, K., Zhou, S. 2019. Iron oxides affect denitrifying bacterial communities with the nirS and nirK genes and potential N2O emission rates from paddy soil. European Journal of Soil Biology, 93, 103093.

Zhou, J., Jiang, X., Wei, D., Zhao, B., Ma, M., Chen, S., Cao, F., Shen, D., Guan, D., Li, J. 2017. Consistent effects of nitrogen fertilization on soil bacterial communities in black soils for two crop seasons in China. Sci Rep, 7(1), 3267.

Zhou, L., Wang, S., Zou, Y., Xia, C., Zhu, G. 2015. Species, abundance and function of ammonia-oxidizing archaea in inland waters across China. Scientific reports, 5(1), 1-13.

Zucker, M., Hankin, L. 1970. Regulation of pectate lyase synthesis in Pseudomonas fluorescens and Erwinia carotovora. Journal of bacteriology, 104(1), 13-18.

Appendix 1

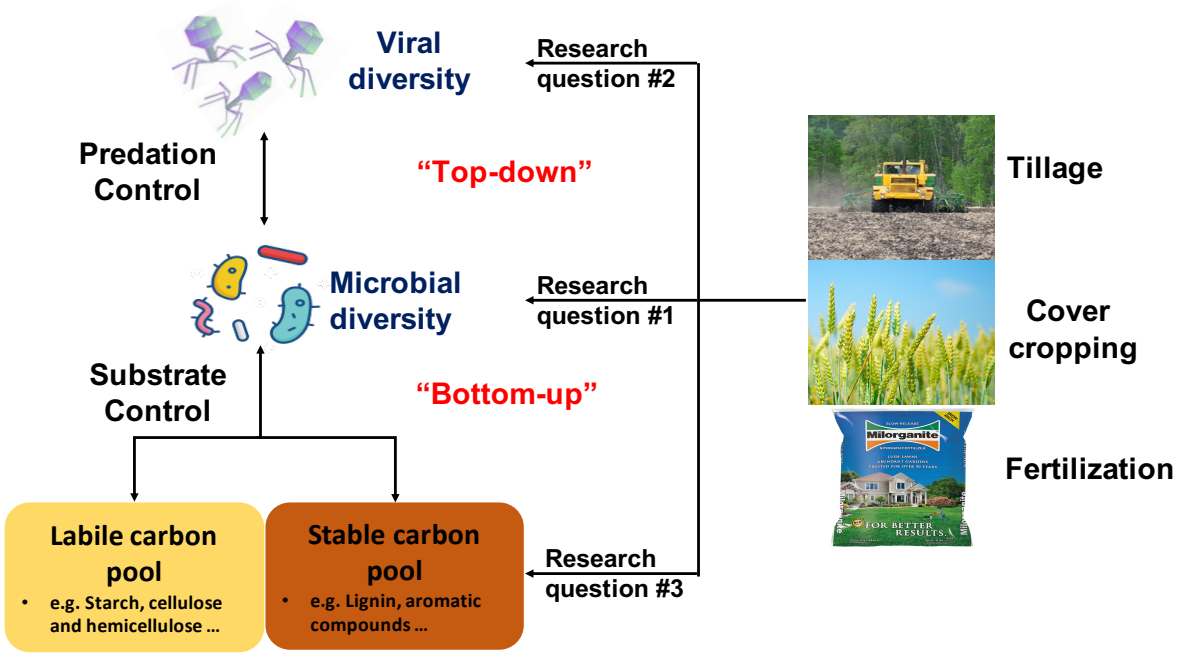


Figure 1.1 Conceptual frame

Chapter 2 Composition of soil viral and bacterial communities after long-term tillage, fertilization, and cover cropping management

A version of this chapter will be originally submitted to Soil Biology and Biochemistry by Ning Duan [a], Lidong Li [b], Xiaolong Liang [c], Regan E. Wagner [a], Aubrey K. Fine [a], Zhibo Cheng [a], Jie Zhuang [a, d], Mark Radosevich [a], Sean M. Schaeffer [a, *]

Contribution statement

Ning Duan carried out the experiment, molecular experiments, and all data processing and statistical analysis. Lidong helped to build a structural equation model. Regan E. Wagner, Aubrey K. Fine provided the viral enumeration data and soil properties data. Xiaolong Liang,and Zhibo Cheng helped with soil sampling and dataset organization. Jie Zhuang, Mark Radosevich, Sean M. Schaeffer are the responsible for the project and help with revision of manuscript.

[a] Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

[b] USDA-Agriculture Research Service (ARS), Agroecosystem Management Research Unit, 255 Filley Hall, 3720 East Campus Loop South, Lincoln, NE 68583, United States

[c] Department of Earth and Planetary Sciences, Washington University in Saint Louis, Saint Louis, MO 63130, United States

d Center for Environmental Biotechnology, The University of Tennessee, Knoxville, TN 37996, United States

* Corresponding author. Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

Abstract

Soil bacterial communities are a critical component for stability and function of terrestrial ecosystems. Viruses are ubiquitous in soils and have significant impacts on the structure and functions of bacterial host communities. However, little is known about the variation of bacterial and viral communities and their connection under various land use management practices. In this study, we evaluated bacterial and viral diversity using 16S rRNA sequencing and RAPD-PCR, respectively, and estimated viral and bacterial abundances by enumeration method, and used structural equation model to reveal relationship between viruses, bacteria, and soil properties, in soils from a long-term conservation management experimental site in western Tennessee USA. Inorganic N fertilization, cover cropping, and tillage treatments showed no significant differences in bacterial alpha-diversity but significantly influenced the structure, as suggested by differences in beta-diversity of bacterial communities. Higher soil pH and water content were favorable to bacterial abundances. Cover cropping, soil water content, and bacterial abundances may explain the variation of viral abundances and community structure in soil. Structural equation modeling showed that bacterial abundances positively influenced viral abundances, and in turn virus abundances and bacterial alpha diversity affected the level of extractable dissolved organic C, which exerted a feedback effect on the structure of bacterial communities. This feedback loop suggests that viral lysates might significantly contribute to reshaping bacterial community structure and indirect influence bacterial alpha diversity. This supports the theory of the "viral shunt" in soil ecosystems. This study indicated that long-term conservation management can reshape soil bacterial and viral communities and influence their interactions in biogeochemical processes.

2.1 Introduction

Soil is a complex ecological system, which contains up to 10^{10} bacteria cells representing thousands of different microbial species in each gram of soil (Raynaud & Nunan, 2014). Viruses, on the other hand, are an understudied component of the soil biosphere that are rapidly gaining the attention of researchers (Kimura et al., 2008a; Emerson et al., 2018; Trubl et al., 2018b; ter Horst et al., 2020). Evidence for viral-mediated top-down regulation of the structure and functions of microbial communities has been reported in soil ecosystems (Ashelford et al., 2003; Williamson et al., 2007; Chow et al., 2014; Emerson et al., 2018; Trubl et al., 2018a). Virus-induced microbial mortality may potentially release dissolved organic matter (DOM) into the rhizosphere. These bioavailable DOM can be readily taken up by microbes. This process is often referred to as the "viral shunt", this definition was come up with in 1999 in marine system, it has been revealed the important role of viruses on C-cycling (Wilhelm & Suttle, 1999; Jover et al., 2014; Kuzyakov & Mason-Jones, 2018). However, the impact of viral shunt on soil microbial food web and nutrient cycling remains unknown. Therefore, the ecological interactions between soil viruses and microbes maybe crucial indicators for functional processes in soil ecosystems (Zablocki et al., 2016).

In agroecosystems, management practices can greatly influence soil properties, including soil structure, carbon sequestration, water and nutrient retention, and microbial distributions in soil profiles. Reduced tillage practices, for example, affect soil water content, temperature, aeration, and the degree of mixing of soil with crop residues, which can reduce soil erosion and increase soil organic matter and water infiltration (Kladivko & Research, 2001; Lienhard et al., 2013).

Fertilizer N input increases soil organic matter, carbon sequestration, and soil acidity (Rousk et al., 2009). Cover cropping can protect soil from erosion and nutrient loss (Romdhane et al., 2019). Both fertilization and cover cropping change the input of crop residues, root exudates, and dynamics of soil physical properties (e.g. moisture and temperature) and in turn influence soil microbial biomass, activity and functional structure (Geisseler & Scow, 2014; Mbuthia, 2014; Vukicevich et al., 2016a; Finney et al., 2017; Zhou et al., 2017). The quality of plant litters from cover crops is, in part, determined by the proportion of C to N. N fixing cover crops (e.g., vetch and clover) have lower C:N ratio and greater labile/recalcitrant C ratio compared to non-N fixing crops (e.g., wheat). The quality of plant litters, as expressed by C:N ratio, will shape microbial communities by selecting of microbes with ability to use ambient resources (Fanin et al., 2016; Vukicevich et al., 2016a). Additionally, root exudate can positively influence microbial biomass because the root exudates contain C-rich substrates, such as amino acids, sugars, and phenolics, for microbial growth (Rasmann et al., 2005; Finney et al., 2017). Soil with no cover cropping typically has a lower buffer capacity for changes in temperature and moisture, resulting in a shift of microbial community to microbes with higher tolerance for environmental fluctuations. For instance, the species with thick cell walls, such as gram-positive bacteria and endospore producing bacteria, can survive harsh soil conditions (Vukicevich et al., 2016a).

In this study, the impacts of long-term conservation management practices on microbial and viral community structure were investigated. We hypothesized that (1) tillage, fertilization, and cover cropping would significalty change the strucutre of bacterial and viral community, compared to conventionally managed control treatments; (2) viral community compostion would be structured in patterns similar to bacterial communities; and (3) viruses may be related to readily organic C

varition and drive the differences in bacterial community structure. To test these hypotheses, high-throughput amplicon sequencing and RAPD-PCR were used to estimate the long-term effect of the management practices on bacterial and viral communities, respectively. Structural equation modeling and multivariate analysis were performed to analyze the interrelationships among bacteria, virus, and environmental factors.

2.2 Materials and methods

2.2.1 Study site and soil sampling

The study site is in the West Tennessee Research and Education Center (WTREC), in Jackson, Tennessee. Soils were collected from treatment plots of a long-term conservation management study producing continuous cotton established in 1981. Calcitic lime was last applied in 2015, and therefore the measured soil pH was typically < 6. The soil in this area is classified as Lexington silt loam (fine-silty, mixed, thermic, Ultic Hapludalf). The experimental field was in a randomized complete block design (RCBD) with split plot design (Fig. S1). In this study, the soil treatments plots sampled from the long-term site included two levels of nitrogen (NH_4NO_3 at 0 and 67 kg ha^{-1}) as the main plots, divided into four sub-plots that contain four kinds of cover crop treatments (hairy vetch, *Vicia villosa* Roth; winter wheat, *Triticum aestivum* L., and no cover). Only the vetch, wheat, and no cover plots were sampled for this study. Each subplot contained two treatments (tillage and no tillage). The collected samples were coded with the following form: “type of cover crop” , “tillage or not”, “fertilization or not” and “serial number of replicates”, e.g. NCCN0_1 represents sampling from the treatment plot of no-cover,

conventional tilled, no nitrogen fertilizer, and replicates 1; VNTN60_4 was sampled in plot with vetch-covered, no-tilled with fertilization, replicates 4; WCN0_3 was sampled in plot with wheat-covered, conventional tillage without fertilization, replicates 3 (Table 2.4).

Soil samples were collected from 0 to 10 cm depth of four replicate plots in each of 12 treatments using a soil probe of 2.5 cm diameter in 2018. About 10-15 samples were randomly taken within each plot, 10-15 cm away from the center of crop row. The subsamples from each plot were thoroughly mixed into one composite sample and then placed into a zip-loc plastic bag. In all, there were 48 samples with 12 treatments and 4 replications of each treatment (Fig. S1). The samples were then transported in a cooler with ice bags within 24 hours to a lab freezer (-20 °C). Small rocks and roots were removed from each soil sample before the soil was passed through 2-mm sieve.

2.2.2 Whole soil DNA extraction, amplification, and sequencing

16S rRNA gene sequencing is widely used for classifying bacterial taxonomy and analyzing bacterial diversity in different environments (Burns et al., 2016; Smets et al., 2016; Orwin et al., 2018; Xia et al., 2019). However, viruses, unlike cellular life, have no universally conserved phylogenetic marker that is comparable to rRNA genes. The methods of characterizing viral communities typically include genetic fingerprinting and metagenomic approaches (Adriaenssens et al., 2015).

DNeasy® PowerLyzer® Powersoil® kit (REF 12855, QIAGEN) was used to extract 48 soil samples, and the DNA was quantified using a Nanodrop (Thermo Scientific NanoDrop ND-3300 Fluorospectrometer 83060-50). The V3-V4 region of 16S rRNA genes was amplified using the published primers (341F 5'- CCTACGGGNGGCWGCAG-3'; 785R 5'-GACTACHVGGGTATCTAATCC-3') yielding a single amplicon of approximately 464 bp (Klindworth et al., 2013). The PCR reaction (25 µL per sample) included 2.5 µL of microbial genomic DNA (5 ng μL^{-1} in 10 mM Tris pH 8.5), 5 µL each of forward and of reverse primer. Platinum Green Hot start master mix (12.5 µL of 2 ×) was used (Invitrogen™, Catalog No. 13001012, U.S.). PCR procedures involved in pre-denaturation at 95˚C for 5 minutes, followed by 25 cycles of denaturation at 95˚C for 40 seconds, annealing at 55˚C for 2 minutes, elongation at 72˚C for 1 minutes, and then held at 4˚C until stored in the freezer (-20 ˚C) for future use. Lastly, the samples were sequenced on an Illumina MiSeq platform with generation of 300 pair-end reads (Bartram et al., 2011) conducted at DNA Genomics Core, University of Tennessee. MiSeq Control Software version 2.6.2.1 was used in this study.

2.2.3 Amplicon sequence data processing

FastQC was performed to test the quality of MiSeq sequencing data (Andrews, 2010) and the filtered sequence data were processed using Mothur v. 1.35.1 (Schloss et al., 2009) according to the MiSeq SOP (https://mothur.org/wiki/miseq_sop/) (Kozich et al., 2013). The contigs with ambiguous bases were screened after the step of *make.contigs* and remaining high-quality contigs were aligned with SILVA bacterial reference database (v.132) using kmer searching. After de-noising the alignment and sequences, we used pre-cluster command split sequences by

group and sorted them by abundance with no more than 1 difference per 100 bp of sequences. Chimeric sequences were removed using the VSERACH program (Rognes et al., 2016). Next, sequences were classified using the Ribosomal Database Project (RDP) Naive Bayesian classifier to reference sequences with cutoff of bootstrap value 80% (Wang et al., 2007). Sequences assigned as Chloroplast, Mitochondria, unknown, Archaea, and Eukaryota were removed. Then sequences with no more than 3% difference were clustered into one OTU and taxonomic table was generated by *make.shared* command for further analysis (Schloss et al., 2009; Schloss, 2020). The results were then visualized in R through RStudio 1.1.463 interface (Team, 2015), using the *phyloseq* (v1.30.0) and *vegan* (v 2.5.7) for community structure analysis, alpha-diversity, beta-diversity and other related analyses. All the data was deposited in the National Center for Biotechnology Information Databases (Sequence Read Archive), under BioProjects accession number is PRJNA744634.

2.2.4 Bacterial diversity and statistical analysis

Alpha-diversity and beta-diversity measurement

The observed richness and evenness values were estimated as number of species, Shannon-Wiener (H'), and the number of OTUs. The Shannon-Wiener index considers species richness and evenness, the number of OTUs indicates the richness of each sample (Kim et al., 2017). All indices were calculated based on the rarefied OTU table.

Canonical Analysis of Principal coordinates (CAP) was conducted to examine the dissimilarities in bacterial and viral community structure among individual samples. A three-way Permutational Multivariate Analysis of Variance (PERMANOVA) test was conducted in R programming language to estimate the significance of community composition differences between two or more treatments (Anderson, 2014).

2.2.5 Viral and bacterial particle extraction and enumeration procedure

The extraction of viral particles and bacterial cells from the soil samples were accomplished using a modified method that was published by Williamson, et al (Williamson et al., 2005b; Williamson et al., 2007; Williamson et al., 2008). Briefly, to extract virus-like particles, 5 g of soil was mixed with 100 mL of 1% potassium citrate in phosphate buffer (1% w/v potassium citrate in phosphate buffer, 1.44 g Na_2HPO_4, 0.24 g K_2HPO_4, 10 g potassium citrate, pH 7.0). The soil slurry was mixed and centrifuged at 5,000 × *g* for 30 minutes at 4°C. The supernatant was filtered through 0.22 µm syringe filters (low protein binding Durapore membrane; Merck Millipore, Cork, Ireland) into 2 mL tubes. The filtered viral extract was flash frozen in liquid nitrogen and stored in -80°C freezer. To extract the bacterial particles from the soil samples, the 100 mL of potassium citrate buffer solution mentioned above was added to 10 g of each soil sample. The soil slurry (1:4.5, v/v) was injected slowly along the side of 15-mL centrifuge tubes onto a layer of 60% (w/v) Nycodenz solution (Cosmo Bio USA, U.S.). The mixture was then centrifuged at 5,000 × *g* for 30 minutes at 4°C using an Accuspin 3R refrigerated centrifuge (Fisher Scientific, Product Code: FS-AS3R). The supernatant including the nycodenz water interface was transferred to a new tube with 1 mL of glycerol (Fisher Chemical™, Catalog No.

G33-500, U.S.) to protect bacteria from freezing stress during flash freezing in liquid nitrogen and stored at -80°C freezer until prepared for enumeration.

The enumerations of viruses and bacteria cells with epifluorescence microscopy followed the steps as reported in (Williamson et al., 2007; Williamson et al., 2008). OPTIZYME™ DNase I (RNase-Free) (Fisher BioReagents™, Catalog No. BP81071, U.S.) was applied to viral and bacterial extracts to remove free DNA. SYBR Gold Dye (2 ×) as DNA-binding dye prepared from the 10 × SYBR gold dye stock solution (Invitrogen™, Catalog No. S11494, U.S.) was used for counting viral-like particles and bacteria using a Nikon Eclipse E600W epi-fluorescence microscope (Nikon, Japan). Images were captured using a Retiga EXi Cooled Mono 12-bit camera (Qimaging, Canada).

2.2.6 RAPD-PCR and gel visualization and analysis

Randomly Amplified Polymorphic DNA-Polymerase chain reaction (RAPD-PCR) provides a low-cost, fingerprinting tool that has been applied to examine the genetic diversity of viruses in aquatic environments (Helton & Wommack, 2009; De Corte et al., 2010; Borrel et al., 2012), sediments (Helton & Wommack, 2009), and soil (Srinivasiah et al., 2013; Liang et al., 2019). Many peer-reviewed studies on viral ecology that have applied RAPD-PCR and first generation sequencing methods (e.g., Sanger sequencing) to evaluate the viral communities (Zablocki et al., 2016). In RAPD-PCR, around 10 nucleotides are used as both forward and reverse primers to amplify viral DNA template in non-specific PCR reactions (Winget & Wommack, 2008).

The virus extract was diluted to approximately 10^5 virus-like particles (VLPs) per mL. Five units of DNase I, 2 µL of 10 × DNase buffer, and 20 µL of viral extract were mixed and incubated at 37 °C for 30 min. Reactions were terminated by addition of 5 µL of DNase I stop solution (50 µM EDTA) and incubated at 65°C for 10 min. Primers (341F and 785R) for bacterial 16S rRNA genes were used for PCR to check the presence of the bacterial genomic DNA in the final viral extracts before RAPD-PCR. All reagents mentioned above were filtered through 0.1 µm filters prior to use.

RAPD-PCR assay mixtures of 25 µL total volume containing 2.5 µL of 10 × Ex Taq Buffer (Mg^{2+}plus), 1.6 µL of dNTP Mixture (0.16mM each), 0.5 µL of TaKaRa Ex Taq HS (5U μl^{-1}) (TaKaRa Bio USA, Catalog No. RR006A, U.S.), 2 µL of primer (5 µM), and 1 µL of viral DNA template, 17.4 µL of nuclease-free water. HCB-1 (5'–CCAGCAGCAG–3') primer was used in all reactions (Srinivasiah et al., 2013). The amplification reaction conditions consisted of denaturation at 94 °C for 10 min followed by primer annealing at 47 °C for 3 min, primer extension at 72 °C for 1 min, and 94 °C for 30 s, and repeated for 32 cycles. The final cycle was followed by primer annealing at 47 °C for 3 min and final primer extension at 72 °C for 10 min, and the temperature was held at 4 °C until further analysis (Winget & Wommack, 2008; Srinivasiah et al., 2013, 2015). RAPD-PCR products (12 µL per sample) were loaded on a gel, consisting of 2% MetaPhor agarose (2g agarose + 100 ml 1× Tris-borate-EDTA, TBE) and electrophoresed at 4 V per cm. The DNA ladder with molecular size 100 bp markers were loaded into lanes (Srinivasiah et al., 2013). Gels were stained with 1 × SYBR gold (Molecular Probes Inc., Eugene, OR) and visualized using an Investigator Eclipse UV Workstation.

Gels were analyzed using a Kodak Gel Logic 100 imaging system. GelCompar II (version 4.5; Applied Maths, Sint-Martens-Latem, Belgium) software was used to analyze banding patterns. The genetic fingerprint pattens compared based on molecular weight (determined by co-migration with standards) and relative intensity of each band. If the difference of two bands size was less than 25 bp, they were considered a single band. The molecular weight of all bands ranged from 1414 to 160. Each band was treated as one "artificial viral OTU", and each sample was assigned a "0" if a band was absent or "1" if the band was present. In this way, an artificial OTU table was generated for downstream analysis.

2.2.7 Soil properties

Soil temperature was measured in the field using temperature probe (Fisherbrand™ Traceable™ Digital Dial Thermometer). The pH was measured using an electrode (Ul-trabasic, Denver Instrument, Bohemia, NY, USA). The water content (GWC) was determined gravimetrically. Salt-extractable dissolved organic carbon (EOC) and microbial biomass carbon (MBC) were measured based upon the chloroform slurry method (Fierer & Schimel, 2002). Briefly, two subsamples from each sample were weighed into glass extraction vessels. Each subsample contained 10 g of fresh soil and one of the subsamples received 1.0 mL ethanol-free chloroform treatment. Then all samples were mixed with 40.0 mL of 0.5 mol L^{-1} K_2SO_4 to the subsamples with chloroform. Samples were placed on a shaker at 150 rpm for 4 hours. Samples were centrifuged and then vacuum filtered with 1.0 μm glass microfiber filters (Whatman). MBC was calculated as the differences between chloroform-exposed and non-chloroform-exposed extractable organic carbon (EOC). Extracts were used for the measurement of NO_3^-, and NH_4^+

concentration using microplate-based spectrophotometric determinations (Rhine et al., 1998; Doane & Horwáth, 2003). Permanganate-oxidizable carbon (POXC) extraction and measurement was conducted using a microplate reader (96-well microplate) at 550 nm was conducted as previously reported (Weil et al., 2003; Culman et al., 2012).

2.2.8 Structural equation modeling

Structural equation modeling (AMOS 27; IBM Corporation, Meadville, PA, USA) was conducted to evaluate the relationships between viral and bacterial abundances and the effects of agricultural management practices and associated soil properties on bacterial and viral abundances. Path coefficients were determined by maximum likelihood estimation at $p \leq 0.05$. Multivariate normality was evaluated according to Kurtosis value ≤ 7. Model fit was evaluated by (1) the minimum discrepancy divided by its degrees of freedom (CMIN/DF) in the range of 1 to 3 (Carmines & McIver, 1983), (2) the goodness of fit index (GFI) close to 1 (Tanaka & Huba, 1985), (3) the comparative fit index (CFI) close to 1 (Bentler, 1990), and (4) the root mean square error of approximation (RMSEA) less than 0.05 (Browne & Cudeck, 1993). We followed the procedures of developing and modifying a structural equation model in Byrne (2013) and Li et al. (2019b). Briefly, we proposed a hypothesized model and test if existing pathways were significant and if necessary pathways were left out. Then, we adjusted the hypothesized model by dropping insignificant pathways and adding missing pathways taking into consideration of model fit and scientific rationality.

2.2.9 Statistical analysis

Mixed Model Analysis of Variance was conducted using SAS software with the Glimmix procedure (SAS Institute Inc., Cary, NC) to test the diversity indices (the number of OTUs, Shannon) under 11 treatments excluding treatment VCN0. The normality, equal variance and outliers were checked using UNIVARIATE procedure. The least square means were compared with Fisher's Least Significant Difference (LSD) at 5% significant level and Tukey's Honest significant difference test were performed to compare differences between different treatments. The RCBD with split-split plot model was used:

$y_{ijkl}=\mu+B_i+F_j+B*F_{ij}+C_k+C*F_{jk}+B*C*F_{ijk}+T_l+T*F_{jl}+T*C_{kl}+T*C*F_{jkl}+B*\ T*C*F_{ijkl}$

Where B is block, F is fertilization, C is cover crops, and T is tillage treatment, and y is indices for measuring alpha-diversity.

2.3 Results

A total of 6,708,766 raw reads were obtained from 44 of 48 soil samples after quality filtering. Four samples, including VCN0_1, VCN0_2, VCN0_3, and VNTN60_4, were removed from further analyses based upon MiSeq SOP due to the low quality. Sequences (4,639,688) with median length of 422 base pairs passed through the quality control process. The number of reads in 44 samples ranged from 38,370 to 398,727 with a mean of 105,447. In total, 118,550 OTUs were classified in 44 samples, and the average number of OTUs in each sample was 10,974. After rarefaction, 20,008 OTU remained with a mean of 3,937 and a range from 3,423 (WNTN0_2) to 4368 (WCN0_4).

The 12 bacterial phyla with a relative abundance greater than 1% included *Proteobacteria*, *Acidobacteria, Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Firmicutes*, *Verrucomicrobia*, *Bacteroidetes*, *Gemmatimonadetes*, *Rokubacteria*, *Patescibacteria*, and *Nitrospirae* (Fig. 2.1). These taxa accounted for more than 95% of the total bacterial relative abundance in each treatment according to results of Type III tests of fixed effects obtained by SAS system Glimmix ANOVA for 12 phyla.

2.3.1 Influences of fertilization, tillage and cover cropping on bacterial diversity

No significant variation in alpha diversity indices across fertilization, tillage, and cover cropping treatments were observed (Fig. 2.2). Canonical Analysis of Principal coordinates (CAP) and the permutational multivariate analysis of variance (PERMANOVA) were conducted based on bacterial communities across samples. The results indicated that fertilization, tillage, cover crop, extractable dissolved organic C (EOC), and leucyl aminopeptidase (LAP) were significant indicators of the variance in the structure of bacterial community (Fig. 2.3 and Table 2.2). The influence of EOC was more significant under no-tillage, fertilization, and vetch than other treatments ($p < 0.05$). Leucine aminopeptidase tended to have greater correlation with the no tillage, no fertilization, and wheat treatment ($p < 0.001$). In total, all environmental variables, along with management treatment, explained approximately 21.79 % of the variations of bacterial community structure.

2.3.2 Influences of tillage, fertilization and cover cropping on viral community composition

RAPD-PCR was used to obtain genetic fingerprints and compare the viral communities in soils under the long-term conservation management practices. The results of PERMANOVA analysis indicated that only cover cropping significantly influenced viral community structure ($p < 0.05$) and could only explain the 1.1% variation of viral communities among soil samples (Fig. 2.4 and Table 2.3).

2.3.3 Correlation among environmental variables, bacterial and viral communities

The effect of soil management on bacterial and viral counts from enumeration and environmental factors was optimally fitted using a structural equation model (CMIN/DF = 0.98, CFI = 1.00, RMSEA = 0.00) (Fig. 2.5 and Table 2.1). The results showed that bacterial abundances and moisture content directly influenced virus abundance, with an effect size of 0.38 and 0.32, respectively. In other words, each 1.00-unit of increase in bacterial abundance or moisture content resulted in 0.38-unit or 0.32-unit increase in viral abundances, respectively. Viral abundances and bacterial alpha diversity represented by Shannon index positively influenced the quantity of EOC. Moisture content and soil pH were the important influencers on bacteria abundances. Vetch could indirectly affect bacterial abundances through influencing moisture content. Tillage negatively influenced POXC with an effect size 0.38.

2.4 Discussion

2.4.1 Bacterial community composition after 40 years of conservation management

Long-term reduced tillage and organic matter input from winter cover crops can potentially affect the composition and functions of soil microbial communities (Wang et al., 2017). However, the results of this study indicate that there were no significant differences in bacterial community richness and evenness (i.e., alpha-diversity) between tillage and no-tillage treatments. One possible reason may be related to the long-term nature of the study site. In other words, it is likely that microbial communities may have adapted and stabilized to a new climax community after the initial establishment of the management practices some 40+ years ago. These new communities are likely dominated by species that have acclimated to the ambient environment conditions after the long-term soil management (DeAngelis et al., 2010).

Bacterial communities are highly responsive to C and/or N addition (Ramirez et al., 2010a). In this study, fertilization, cover cropping, and tillage involved in C and N inputs were significant factors influencing the structure of bacteria communities. Hairy Vetch with low C:N ratio (11:1), that contained starch, cellulose, and other carbon substrates under fertilization, may promote secretion of extracellular enzymes and increase salt-extractable dissolved organic C component (Sievers & Cook, 2018). The increasing of the bacterial diversity under the dissolved organic matter amended cultures were observed (Landa et al., 2013), which is consistent with the result that positive influence of bacterial alpha diversity on quantity of EOC in this study. Surface soil without tillage contained significantly greater dissolved organic C than conventional tillage soil

(Linn & Doran, 1984c; Liu et al., 2014). This supports our results revealed by the SEM model that tillage has a negative effect on POXC component. Production and activities of extracellular enzymes of microbes can be stimulated by availability and quality of soil organic matter (de Almeida et al., 2015; Loeppmann et al., 2016). Increased leucyl aminopeptidase may explained that soil peptides were decomposed by aminopeptidase to full fill consumption of C substrate by bacteria like wheat residue with high C:N ratio (Vaughan & Malcolm, 2012).

Other environmental factors, such as soil water content and pH, can directly influence bacterial abundances (Stres et al., 2008b; Zhalnina et al., 2015a; Wan et al., 2020). Bacterial growth is positively correlated with soil pH ranging from 4 to 8 in arable soil (Rousk et al., 2010c). The increase in soil water content promoted bacterial abundances, which may be related to increases in the diffusion of soluble substrate and oxygen that are favorable for bacterial growth (Stres et al., 2008b).

2.4.2 Viral diversity patterns in conservation management systems

The soil virus community varied due to soil properties (e.g., water content and organic C input) induced by cover cropping. The spatial distribution of viruses mainly depends on the passive movement of viral particles due to their light weight and small volume. Movement of viral particles occurred in liquid phase are primarily driven by a change of hydraulic gradient and sorption to soil matrix by electrostatic double-layer interactions, hydrophobic interactions, and van der Waals forces (Goyal & Gerba, 1979; Gerba, 1984; Tim & Mostaghimi, 1991; Zhuang & Jin, 2003b; Kenst et al., 2008). Elevated soil moisture could not only facilitate virus mobility in

the soil micro-habitat and increase infection rates (Hurst et al., 1980b; Armanious et al., 2016; Kuzyakov & Mason-Jones, 2018), but also increase release of protected organic matter into more biologically accessible soil pores (Huang & Hall, 2017). Dissolved organic matter could decrease the interfacial tension at the air-water interface and increase the survival of hydrophobic viruses at air-water surface (Powelson et al., 1991). In addition, organic matter with a charge could compete with viral adsorption by blocking adsorption sites on soil particles, and this would facilitate virus distribution and increase bacterial infection (Powelson et al., 1991; Zhuang & Jin, 2003a). Organic matter can decrease adsorption of poliovirus to soil (Lance & Gerba, 1984). Very few similar studies have been conducted with native soil viruses. Different types of viruses with different charges due to the distinct components of viral proteins in capsids (Karlin & Brendel, 1988) and physical and chemical properties of organic material also determined the transport and distribution of viruses in soil (Zhuang & Jin, 2003a). In this study, the observed increase in viral abundance was attributed to moisture increase and organic carbon and N availability as a result of cover cropping. Although our results did not show labile C influence on viral abundances, dissolved humic acid involved in competition of virus sorption sites can reflect changes in viral adsorption on soil particles (Zhuang & Jin, 2003a). Therefore, the influence of soil moisture and organic matter composition on viruses should be considered in the interpretation of the variation of viral communities.

2.4.3 Association between bacterial and viral community

We found that viral abundance is positively related to bacterial abundance. Viral burst size, viral production, and rate of bacterial cell lysis positively depended on the steady-state growth rate of

bacteria in a specific viral-bacteria system. Also, the growth of non-infected and resistant bacterial cells were stimulated by viral lysates (Middelboe, 2000). Viruses have impact on bacterial abundances. For example, the growth of *Vibrio natriegens* were strongly restrained by increasing phage infection rate in lab experiments (Hennes et al., 1995). The competitive bacterial species with dominated abundance and activity (superior competitors) might trigger a net positive lytic viral production ('kill the winner' mechanism) to control their abundance (Thingstad & Lignell, 1997; Weinbauer & Rassoulzadegan, 2004a).

In addition, we found that bacteria abundances directly and positively influence viral abundance, bacterial alpha diversity and viral abundance have a positive effect on soil EOC. At the same time, the structure of bacterial communities was driven by EOC content, suggesting that readily organic C received direct influence from bacterial diversity, as well as viral lysis, which can shift the bacterial communities. The readily organic C might be due to release from bacterial cells through viral lysis, which might play a role in shifting bacterial community structure and affect bacterial richness and evenness based on the availability of labile carbon substrates and mortality of infected taxa (Wilhelm & Suttle, 1999; Weinbauer & Rassoulzadegan, 2004a). The relationship between viral and bacterial communities observed in this study may support "viral shunt" concept in the soil environment (Williamson et al., 2017a).

Together, virus-bacteria interaction is not a simple unidirectional relationship in soil, but a relationship strongly influenced by resource availability from viral lysates, bacterial status (resistance, infected, or uninfected), viral infection, and bacterial growth. These results provide

the evidence for a close relationship between virus and bacterial abundance and diversity in agricultural soil systems.

2.5 Conclusions

Long-term tillage, fertilization, and cover cropping management practices contribute to the structure of bacterial communities. Vetch, with its low C:N ratio, may promote the production of EOC, while wheat, with higher C:N ratio, could stimulate N-cycling enzymes, such as leucine aminopeptidase for matching the extra C consumption. The organic matter component from different cover cropping treatments and different moisture contents might alter the mechanism of adsorption or distribution of viruses and differentiate the viral communities. Greater soil water content in vetch treatment would increase the bacterial and viral abundances. Our results indicate that increases in viral abundance potentially promote release of readily available carbon possibly originating from lysed host cells, resulting in increased EOC. The increasing of EOC content can result in bacterial alpha diversity increased. This bioavailable C could then be taken up and metabolized by surrounding bacteria, reshaping the bacterial communities. However, the RAPD-PCR approach used here to assess differences in viral genetic diversity suffers from low resolution and lack of taxonomic information to explore the correlation between virus and bacteria communities at genetic level. Further study with more robust metagenomic approaches should be conducted to reveal the ecological role of viruses more accurately in the agroecosystems.

References

Adriaenssens, E. M., Van Zyl, L., De Maayer, P., Rubagotti, E., Rybicki, E., Tuffin, M., Cowan, D. A. 2015. Metagenomic analysis of the viral community in Namib Desert hypoliths. Environ Microbiol, 17(2), 480-495.

Anderson, M. J. 2014. Permutational multivariate analysis of variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online, 1-15.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data.

Armanious, A., Aeppli, M., Jacak, R., Refardt, D., Sigstam, T., Kohn, T., Sander, M. 2016. Viruses at solid–water interfaces: a systematic assessment of interactions driving adsorption. Environmental science & technology, 50(2), 732-743.

Ashelford, K. E., Day, M. J., Fry, J. C. 2003. Elevated abundance of bacteriophage infecting bacteria in soil. Applied and environmental microbiology, 69(1), 285-289.

Bentler, P. M. 1990. Comparative fit indexes in structural models. Psychological bulletin, 107(2), 238.

Borrel, G., Colombet, J., Robin, A., Lehours, A.-C., Prangishvili, D., Sime-Ngando, T. 2012. Unexpected and novel putative viruses in the sediments of a deep-dark permanently anoxic freshwater habitat. The ISME journal, 6(11), 2119-2127.

Browne, M. W., Cudeck, R. 1993. Alternative ways of assessing model fit In: Bollen KA, Long JS, editors. Testing structural equation models. Beverly Hills, CA: Sage, 111-135.

Burns, K. N., Bokulich, N. A., Cantu, D., Greenhut, R. F., Kluepfel, D. A., O'Geen, A. T., Strauss, S. L., Steenwerth, K. L. 2016. Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by vineyard management. Soil Biology and Biochemistry, 103, 337-348.

Byrne, B. M. 2013. Structural equation modeling with AMOS: Basic concepts, applications, and programming (2nd ed.): New York: Routledge.

Carmines, E. G., McIver, J. P. 1983. An introduction to the analysis of models with unobserved variables. Political Methodology, 51-102.

Chow, C. E. T., Kim, D. Y., Sachdeva, R., Caron, D. A., Fuhrman, J. A. 2014. Top-down controls on bacterial community structure: microbial network analysis of bacteria, T4-like viruses and protists. The ISME journal, 8(4), 816-829.

Culman, S., Freeman, M., Snapp, S. 2012. Procedure for the determination of permanganate oxidizable carbon. Hickory Corners MI: Kellogg Biological Station.

de Almeida, R. F., Naves, E. R., da Mota, R. P. 2015. Soil quality: Enzymatic activity of soil β-glucosidase. Global Journal of Agricultural Research and Reviews, 3(2), 146-150.

De Corte, D., Sintes, E., Winter, C., Yokokawa, T., Reinthaler, T., Herndl, G. J. 2010. Links between viral and prokaryotic communities throughout the water column in the (sub) tropical Atlantic Ocean. The ISME journal, 4(11), 1431-1442.

DeAngelis, K. M., Silver, W. L., Thompson, A. W., Firestone, M. K. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental microbiology, 12(12), 3137-3149.

Doane, T. A., Horwáth, W. R. 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical letters, 36(12), 2713-2722.

Emerson, J. B., Roux, S., Brum, J. R., Bolduc, B., Woodcroft, B. J., Jang, H. B., Singleton, C. M., Solden, L. M., Naas, A. E., Boyd, J. A. 2018. Host-linked soil viral ecology along a permafrost thaw gradient. Nature Microbiology, 3(8), 870-880.

Fanin, N., Fromin, N., Bertrand, I. 2016. Functional breadth and home-field advantage generate functional differences among soil microbial decomposers. Ecology, 97(4), 1023-1037.

Fierer, N., Schimel, J. P. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry, 34(6), 777-787.

Finney, D. M., Buyer, J. S., Kaye, J. P. 2017. Living cover crops have immediate impacts on soil microbial community structure and function. Journal of Soil and Water Conservation, 72(4), 361-373.

Geisseler, D., Scow, K. M. 2014. Long-term effects of mineral fertilizers on soil microorganisms–A review. Soil Biology and Biochemistry, 75, 54-63.

Gerba, C. P. 1984. Applied and theoretical aspects of virus adsorption to surfaces. Advances in applied microbiology, 30, 133-168.

Goyal, S. M., Gerba, C. P. 1979. Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. Applied and environmental microbiology, 38(2), 241-247.

Helton, R. R., Wommack, K. E. 2009. Seasonal dynamics and metagenomic characterization of estuarine viriobenthos assemblages by randomly amplified polymorphic DNA PCR. Appl Environ Microbiol, 75(8), 2259-2265.

Hennes, K. P., Suttle, C. A., Chan, A. M. 1995. Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. Applied and environmental microbiology, 61(10), 3623-3627.

Huang, W., Hall, S. J. 2017. Elevated moisture stimulates carbon loss from mineral soils by releasing protected organic matter. Nature Communications, 8(1), 1-10.

Hurst, C. J., Gerba, C. P., Cech, I. 1980. Effects of environmental variables and soil characteristics on virus survival in soil. Applied and environmental microbiology, 40(6), 1067-1079.

Jover, L. F., Effler, T. C., Buchan, A., Wilhelm, S. W., Weitz, J. S. 2014. The elemental composition of virus particles: implications for marine biogeochemical cycles. Nature Reviews Microbiology, 12(7), 519-528.

Karlin, S., Brendel, V. 1988. Charge configurations in viral proteins. Proceedings of the National Academy of Sciences, 85(24), 9396-9400.

Kenst, A. B., Perfect, E., Wilhelm, S. W., Zhuang, J., McCarthy, J. F., McKay, L. D. 2008. Virus transport during infiltration of a wetting front into initially unsaturated sand columns. Environmental science & technology, 42(4), 1102-1108.

Kim, B. R., Shin, J., Guevarra, R. B., Lee, J. H., Kim, D. W., Seol, K. H., Lee, J. H., Kim, H. B., Isaacson, R. E. 2017. Deciphering Diversity Indices for a Better Understanding of Microbial Communities. Journal of Microbiology and Biotechnology, 27(12), 2089-2093.

Kimura, M., Jia, Z., Jun, Nakayama, N., Asakawa, S. 2008. Ecology of viruses in soils: past, present and future perspectives. Soil Science and Plant Nutrition, 54(1), 1-32.

Kladivko, E. J. J. S., Research, T. 2001. Tillage systems and soil ecology. 61(1-2), 61-76.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F. O. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic acids research, 41(1), e1-e1.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., Schloss, P. D. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon

sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology, 79(17), 5112-5120.

Kuzyakov, Y., Mason-Jones, K. 2018. Viruses in soil: Nano-scale undead drivers of microbial life, biogeochemical turnover and ecosystem functions. Soil Biology & Biochemistry, 127, 305-317.

Lance, J., Gerba, C. P. 1984. Effect of ionic composition of suspending solution on virus adsorption by a soil column. Applied and environmental microbiology, 47(3), 484-488.

Li, L., Wilson, C. B., He, H., Zhang, X., Zhou, F., Schaeffer, S. M. 2019. Physical, biochemical, and microbial controls on amino sugar accumulation in soils under long-term cover cropping and no-tillage farming. Soil Biology and Biochemistry, 135, 369-378.

Liang, X., Zhuang, J., Loffler, F. E., Zhang, Y., DeBruyn, J. M., Wilhelm, S. W., Schaeffer, S. M., Radosevich, M. 2019. Viral and bacterial community responses to stimulated Fe(III)-bioreduction during simulated subsurface bioremediation. Environ Microbiol, 21(6), 2043-2055.

Lienhard, P., Tivet, F., Chabanne, A., Dequiedt, S., Lelièvre, M., Sayphoummie, S., Leudphanane, B., Prévost-Bouré, N. C., Séguy, L., Maron, P.-A. J. A. f. s. d. 2013. No-till and cover crops shift soil microbial abundance and diversity in Laos tropical grasslands. 33(2), 375-384.

Linn, D. M., Doran, J. W. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal, 48(6), 1267-1272.

Liu, E., Teclemariam, S. G., Yan, C., Yu, J., Gu, R., Liu, S., He, W., Liu, Q. 2014. Long-term effects of no-tillage management practice on soil organic carbon and its fractions in the northern China. Geoderma, 213, 379-384.

Loeppmann, S., Semenov, M., Blagodatskaya, E., Kuzyakov, Y. 2016. Substrate quality affects microbial-and enzyme activities in rooted soil. Journal of Plant Nutrition and Soil Science, 179(1), 39-47.

Mbuthia, L. W. 2014. Long-Term Impacts of Tillage, Cover Crops, and Nitrogen Rates on Microbial Community Dynamics and Soil Quality Parameters under Continuous Cotton Production in West Tennessee.

Middelboe, M. 2000. Bacterial growth rate and marine virus–host dynamics. Microbial Ecology, 40(2), 114-124.

Orwin, K., Dickie, I., Holdaway, R., Wood, J. 2018. A comparison of the ability of PLFA and 16S rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions. Soil Biology and Biochemistry, 117, 27-35.

Powelson, D. K., Simpson, J. R., Gerba, C. P. 1991. Effects of organic matter on virus transport in unsaturated flow. Applied and environmental microbiology, 57(8), 2192-2196.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., Fierer, N. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology, 91(12), 3463-3470.

Rasmann, S., Kollner, T. G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., Turlings, T. C. J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature, 434(7034), 732-737.

Raynaud, X., Nunan, N. 2014. Spatial ecology of bacteria at the microscale in soil. PLoS One, 9(1), e87217.

Rhine, E. D., Mulvaney, R. L., Pratt, E. J., Sims, G. K. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Science Society of America Journal, 62(2), 473-480.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F. J. P. 2016. VSEARCH: a versatile open source tool for metagenomics. 4, e2584.

Romdhane, S., Spor, A., Busset, H., Falchetto, L., Martin, J., Bizouard, F., Bru, D., Breuil, M. C., Philippot, L., Cordeau, S. 2019. Cover Crop Management Practices Rather Than Composition of Cover Crop Mixtures Affect Bacterial Communities in No-Till Agroecosystems. Frontiers in Microbiology, 10.

Rousk, J., Brookes, P. C., Baath, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol, 75(6), 1589-1596.

Rousk, J., Brookes, P. C., Bååth, E. 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology and Biochemistry, 42(6), 926-934.

Schloss, P. D. 2020. Reintroducing mothur: 10 years later. Applied and environmental microbiology, 86(2).

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J. 2009. Introducing mothur: open-source, platform-independent, community-supported software for

describing and comparing microbial communities. Applied and environmental microbiology, 75(23), 7537-7541.

Sievers, T., Cook, R. L. 2018. Aboveground and root decomposition of cereal rye and hairy vetch cover crops. Soil Science Society of America Journal, 82(1), 147-155.

Smets, W., Leff, J. W., Bradford, M. A., McCulley, R. L., Lebeer, S., Fierer, N. 2016. A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. Soil Biology and Biochemistry, 96, 145-151.

Srinivasiah, S., Lovett, J., Polson, S., Bhavsar, J., Ghosh, D., Roy, K., Fuhrmann, J. J., Radosevich, M., Wommack, K. E. 2013. Direct assessment of viral diversity in soils by random PCR amplification of polymorphic DNA. Appl Environ Microbiol, 79(18), 5450-5457.

Stres, B., Danevčič, T., Pal, L., Fuka, M. M., Resman, L., Leskovec, S., Hacin, J., Stopar, D., Mahne, I., Mandic-Mulec, I. 2008. Influence of temperature and soil water content on bacterial, archaeal and denitrifying microbial communities in drained fen grassland soil microcosms. FEMS microbiology ecology, 66(1), 110-122.

Tanaka, J. S., Huba, G. J. 1985. A fit index for covariance structure models under arbitrary GLS estimation. British Journal of Mathematical and Statistical Psychology, 38(2), 197-201.

Team, R. (2015). R-Studio: integrated development for R. R-Studio, Inc., Boston, MA, USA. In.

Ter Horst, A. M., Santos-Medellín, C., Sorensen, J. W., Zinke, L. A., Wilson, R. M., Johnston, E. R., Trubl, G. G., Pett-Ridge, J., Blazewicz, S. J., Hanson, P. J. 2020. Minnesota peat viromes reveal terrestrial and aquatic niche partitioning for local and global viral populations. bioRxiv.

Thingstad, T. F., Lignell, R. 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquatic microbial ecology, 13(1), 19-27.

Tim, U. S., Mostaghimi, S. 1991. Model for predicting virus movement through soils. Groundwater, 29(2), 251-259.

Trubl, G., Jang, H. B., Roux, S., Emerson, J. B., Solonenko, N., Vik, D. R., Solden, L., Ellenbogen, J., Runyon, A. T., Bolduc, B. 2018a. Soil viruses are underexplored players in ecosystem carbon processing. Msystems, 3(5).

Trubl, G., Jang, H. B., Roux, S., Emerson, J. B., Solonenko, N., Vik, D. R., Solden, L., Ellenbogen, J., Runyon, A. T., Bolduc, B., Woodcroft, B. J., Saleska, S. R., Tyson, G. W., Wrighton, K. C., Sullivan, M. B., Rich, V. I. 2018b. Soil Viruses Are Underexplored Players in Ecosystem Carbon Processing. Msystems, 3(5).

Vaughan, D., Malcolm, R. 2012. Soil organic matter and biological activity (Vol. 16): Springer Science & Business Media.

Vukicevich, E., Lowery, T., Bowen, P., Urbez-Torres, J. R., Hart, M. 2016. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development, 36(3).

Wan, W., Tan, J., Wang, Y., Qin, Y., He, H., Wu, H., Zuo, W., He, D. 2020. Responses of the rhizosphere bacterial community in acidic crop soil to pH: Changes in diversity, composition, interaction, and function. Science of the total environment, 700, 134418.

Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and environmental microbiology, 73(16), 5261-5267.

Wang, Y., Li, C., Tu, C., Hoyt, G. D., DeForest, J. L., Hu, S. 2017. Long-term no-tillage and organic input management enhanced the diversity and stability of soil microbial community. Science of the total environment, 609, 341-347.

Warton, D. I., Wright, S. T., Wang, Y. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution, 3(1), 89-101.

Weil, R. R., Islam, K. R., Stine, M. A., Gruver, J. B., Samson-Liebig, S. E. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture, 3-17.

Weinbauer, M. G., Rassoulzadegan, F. 2004. Are viruses driving microbial diversification and diversity? Environ Microbiol, 6(1), 1-11.

Wilhelm, S. W., Suttle, C. A. 1999. Viruses and nutrient cycles in the sea: viruses play critical roles in the structure and function of aquatic food webs. BioScience, 49(10), 781-788.

Williamson, K. E., Fuhrmann, J. J., Wommack, K. E., Radosevich, M. 2017. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. Annual review of virology, 4, 201-219.

Williamson, K. E., Radosevich, M., Smith, D. W., Wommack, K. E. 2007. Incidence of lysogeny within temperate and extreme soil environments. Environmental microbiology, 9(10), 2563-2574.

Williamson, K. E., Radosevich, M., Wommack, K. E. 2005. Abundance and diversity of viruses in six Delaware soils. Applied and environmental microbiology, 71(6), 3119-3125.

Williamson, K. E., Schnitker, J. B., Radosevich, M., Smith, D. W., Wommack, K. E. 2008. Cultivation-based assessment of lysogeny among soil bacteria. Microbial Ecology, 56(3), 437-447.

Winget, D. M., Wommack, K. E. 2008. Randomly amplified polymorphic DNA PCR as a tool for assessment of marine viral richness. Appl Environ Microbiol, 74(9), 2612-2618.

Xia, X., Zhang, P., He, L., Gao, X., Li, W., Zhou, Y., Li, Z., Li, H., Yang, L. 2019. Effects of tillage managements and maize straw returning on soil microbiome using 16S rDNA sequencing. Journal of integrative plant biology, 61(6), 765-777.

Zablocki, O., Adriaenssens, E. M., Cowan, D. 2016. Diversity and Ecology of Viruses in Hyperarid Desert Soils. Appl Environ Microbiol, 82(3), 770-777.

Zhalnina, K., Dias, R., de Quadros, P. D., Davis-Richardson, A., Camargo, F. A., Clark, I. M., McGrath, S. P., Hirsch, P. R., Triplett, E. W. 2015. Soil pH determines microbial diversity and composition in the park grass experiment. Microbial Ecology, 69(2), 395-406.

Zhou, J., Jiang, X., Wei, D., Zhao, B., Ma, M., Chen, S., Cao, F., Shen, D., Guan, D., Li, J. 2017. Consistent effects of nitrogen fertilization on soil bacterial communities in black soils for two crop seasons in China. Sci Rep, 7(1), 3267.

Zhuang, J., Jin, Y. 2003a. Virus retention and transport as influenced by different forms of soil organic matter. Journal of environmental quality, 32(3), 816-823.

Zhuang, J., Jin, Y. 2003b. Virus retention and transport through Al-oxide coated sand columns: effects of ionic strength and composition. Journal of Contaminant Hydrology, 60(3-4), 193-209.

Appendix 2

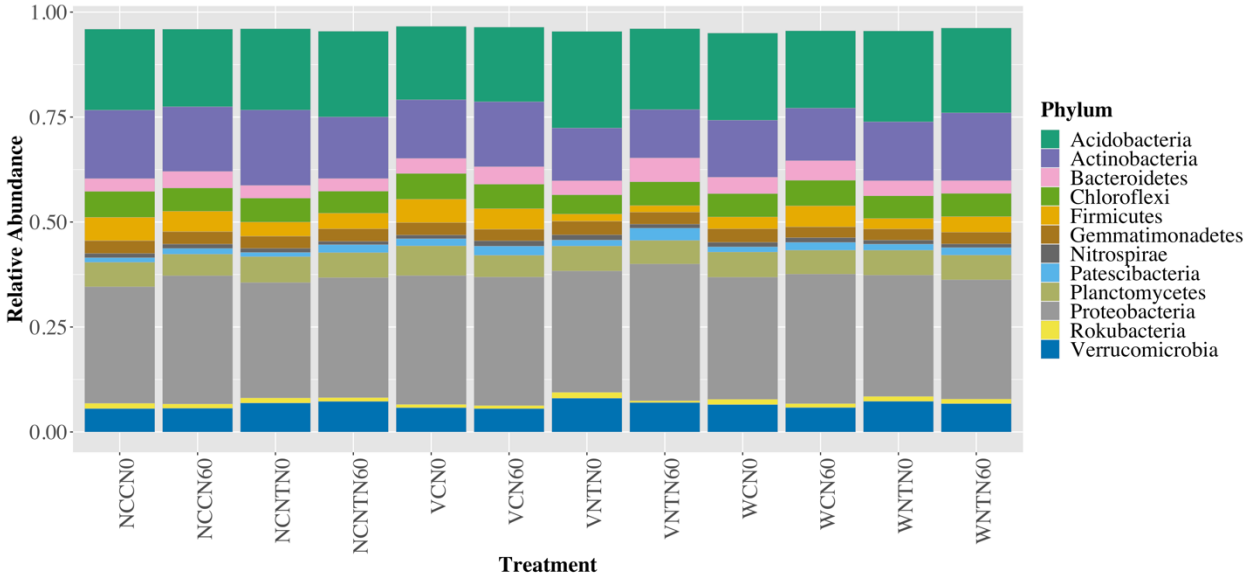


Figure 2.1 The relative abundance of bacteria at phylum level

The phyla with relative abundance over 1% were selected and the 12 soil management treatments are shown in the x-axis, VCN0 treatment only contains 1 sample, VNTN60 has three replicates, all the other 10 treatments have 4 replicates. The relative abundance was derived from the mean of four biological replicates except for the VCN0 and VNTN60 treatment.

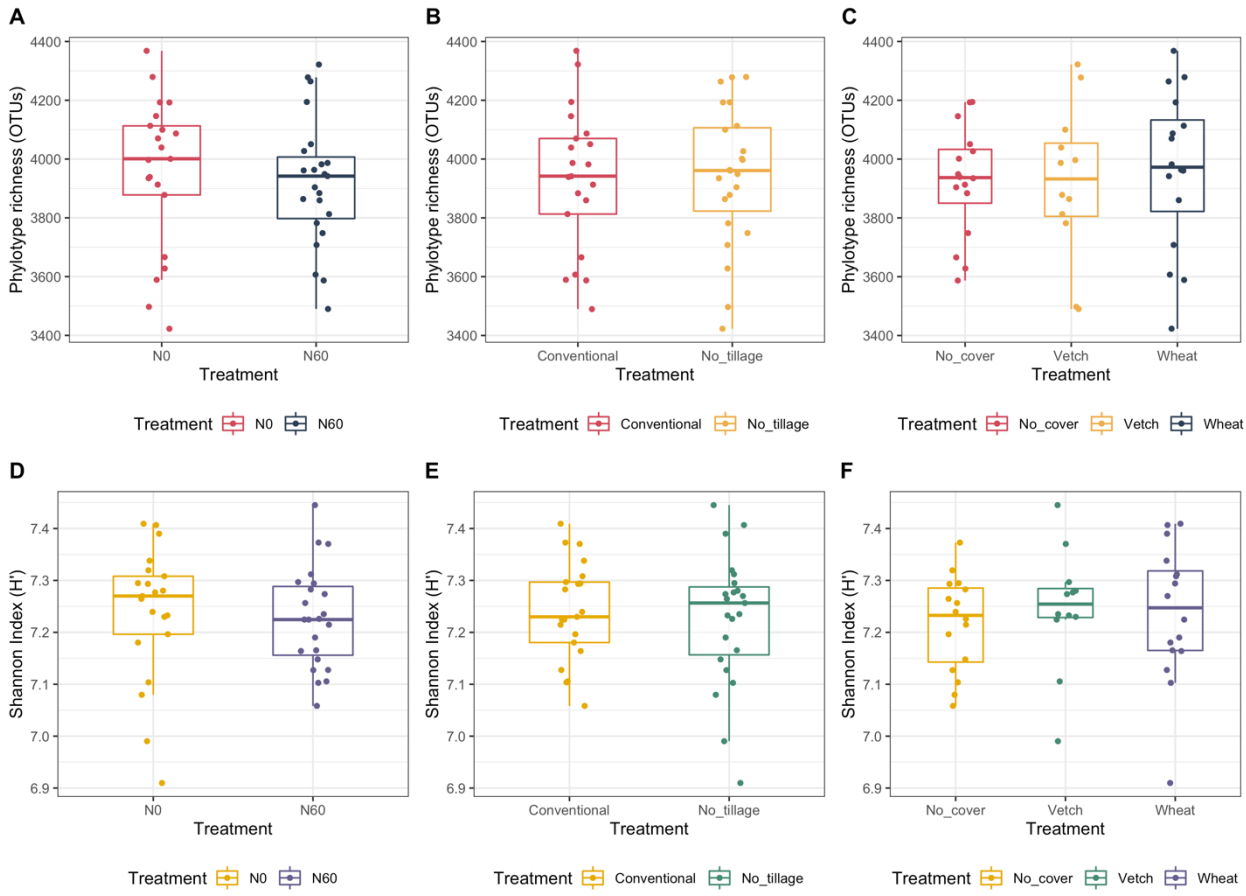


Figure 2.2 Alpha diversity of bacteria communities across fertilization, tillage, and cover cropping.

Richness (1A to 1C) and Shannon index (1D to 1E) of bacterial community across fertilization (1A and 1D), conventional tillage (1B and 1E), and cover cropping 1C and 1F), respectively. The top, middle, bottom-line position in boxes represents the first, median and third quartiles, whiskers extend no more than 1.5 times of inter-quartile range.

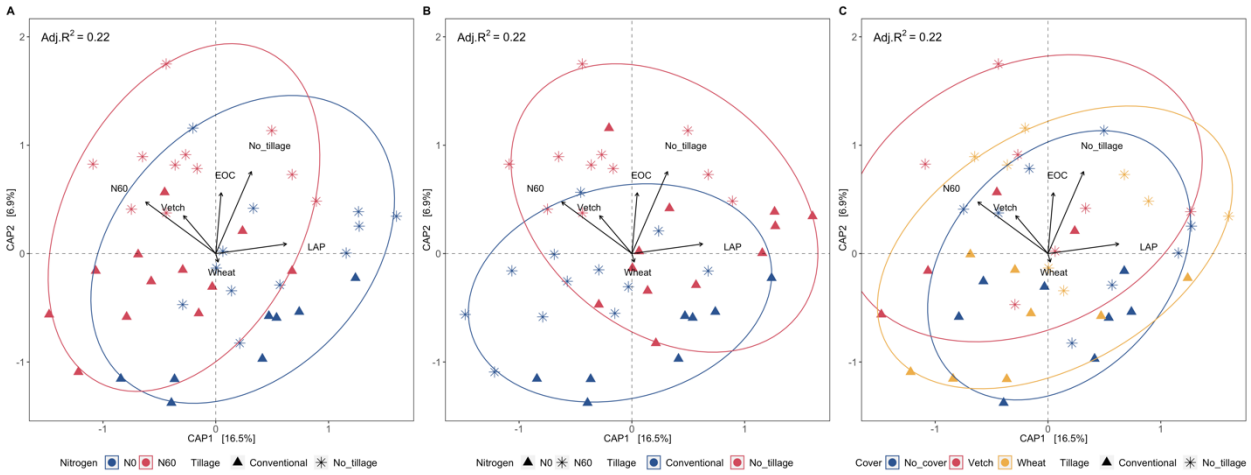


Figure 2.3 Canonical analysis of principal coordinates (CAP) of bacterial communties and environmental factors

The analysis was conducted using Bray-Curtis dissimilarity and grouped by fertilization treatment (A), tillage treatment (B), and cover cropping treatment (C). The directions of arrows in the ordination space represent the direction of maximum change in each variable. The length of an arrow is the importance of an environmental variable. “Virus” represents the viral abundances across samples, significant indicators, tillage (No-tillage) ($p < 0.01$), inorganic N fertilization ($p < 0.001$), cover crop (vetch, wheat) ($p < 0.05$), extractable dissolved organic C (EOC) ($p < 0.05$), and leucyl aminopeptidase (LAP) ($p < 0.001$) were shown as vectors. Adjusted R-squared is the total variation of bacterial community explained by the variables presented as arrows.

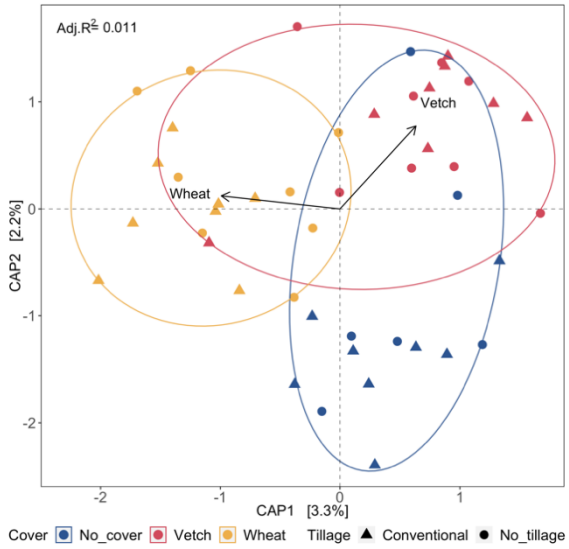


Figure 2.4 Canonical analysis of principal coordinates (CAP) of viral communities across treatments.

Canonical Analysis of Principal coordinates (CAP) of viral communities based upon RAPD-PCR results. The analysis was conducted by Jaccard dissimilarity grouped by cover cropping treatment. The arrow represented the significant factor ($p < 0.05$) that can explain the variation of viral communities. Adjusted R-squared is the total variation of viral community explained by different cover croppinge.

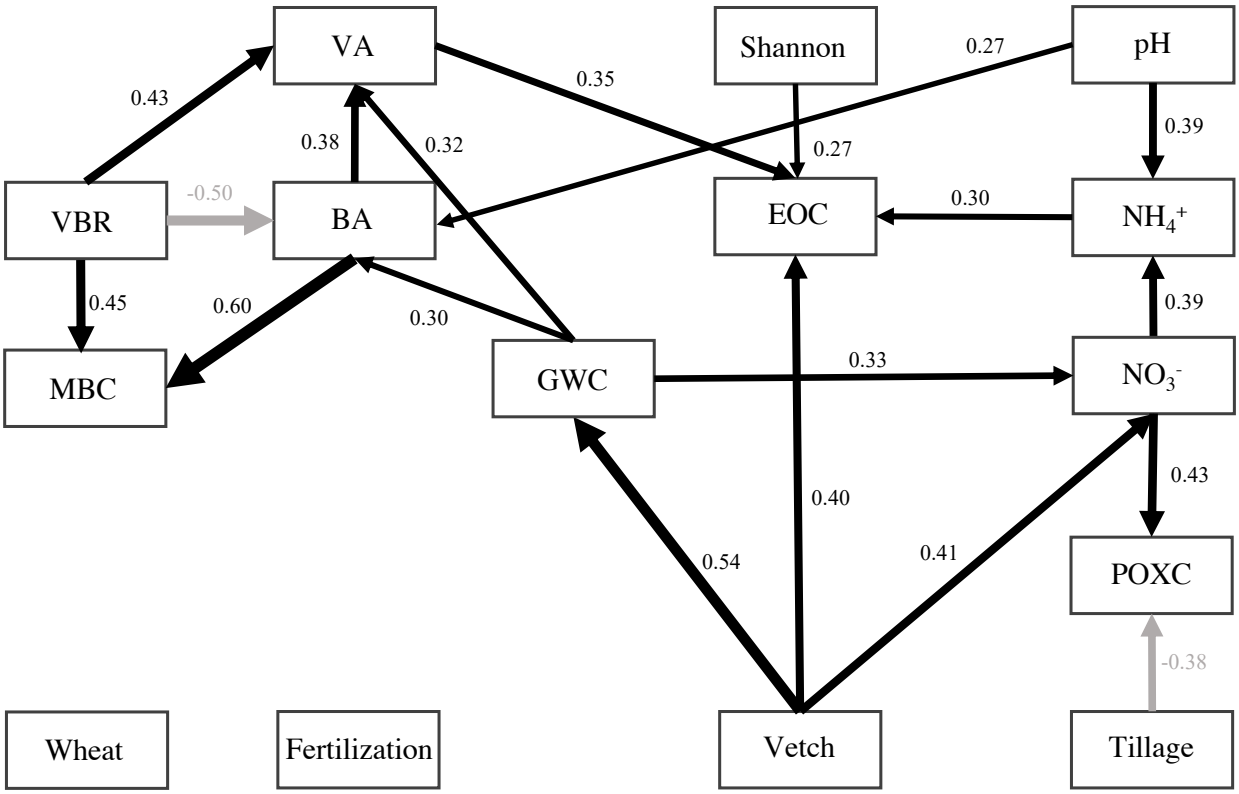


Figure 2.5 Structural equation modeling for relationship among viral, bacteria abundances, and environmental factors.

Boxes represent variables. Single-headed arrows represent causal relationships, and double-headed arrows correlations. Black arrows indicate positive effects, and grey arrows negative effects. No arrows suggest no significant relationships between variables. All presented relationships are significant at $p < 0.05$. Numbers beside each arrow are standardized path coefficients (i.e., effect sizes). See Table 1 for standardized total effects. The "VA", "BA", and "VBR" were presenting the viral, bacterial abundances obtained by enumeration, and viral to bacteria ratio, respectively. Soil pH (pH), extractable dissolved organic C (EOC), leucyl aminopeptidase (LAP), microbial biomass C (MBC), Gravimetric water content (GWC), permanganate-oxidizable carbon (POXC), ammonia (NH_4^+), and nitrate (NO_3^-) were as parameter presented in rectangular box.

Table 2.1 Standardized total effects of structural equation model.

	Vetch	GWC	pH	VBR	NO^{3-}-N	BA	Tillage	NH_4^+-N	Shannon	VA
GWC	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NO^{3-}-N	0.59	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BA	0.16	0.30	0.27	-0.50	0.00	0.00	0.00	0.00	0.00	0.00
NH_4^+-N	0.23	0.13	0.39	0.00	0.39	0.00	0.00	0.00	0.00	0.00
VA	0.23	0.43	0.10	0.24	0.00	0.38	0.00	0.00	0.00	0.00
MBC	0.10	0.18	0.16	0.15	0.00	0.60	0.00	0.00	0.00	0.00
EOC	0.55	0.19	0.15	0.09	0.12	0.13	0.00	0.30	0.27	0.35
POXC	0.25	0.14	0.00	0.00	0.43	0.00	-0.38	0.00	0.00	0.00

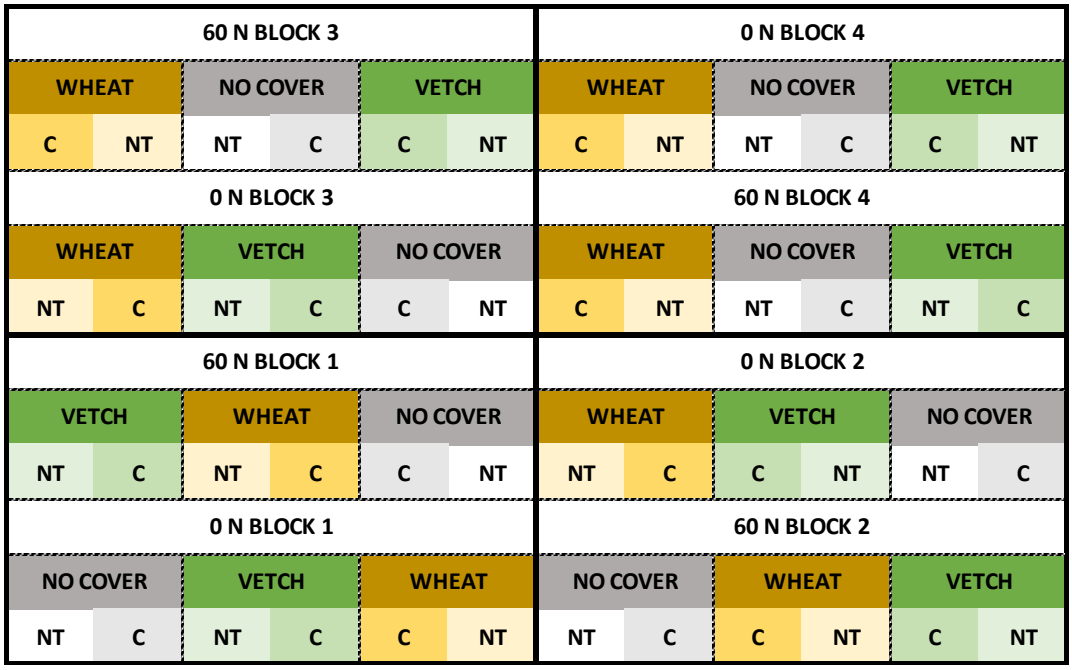


Figure 2.6 Field map.

The plots are 40’ x 26.6’ with 20’ alleys. Total average with alleys 4.5 ac, main plot: nitrogen fertilization rate (0, 60lbs N / ac), Sub-Plot: Cover Crop, No-Cover, Hairy Vetch, Crimson Clover, Wheat. Sub-Sub Plot: Tillage, C= Conventional Till, NT= No-Till.

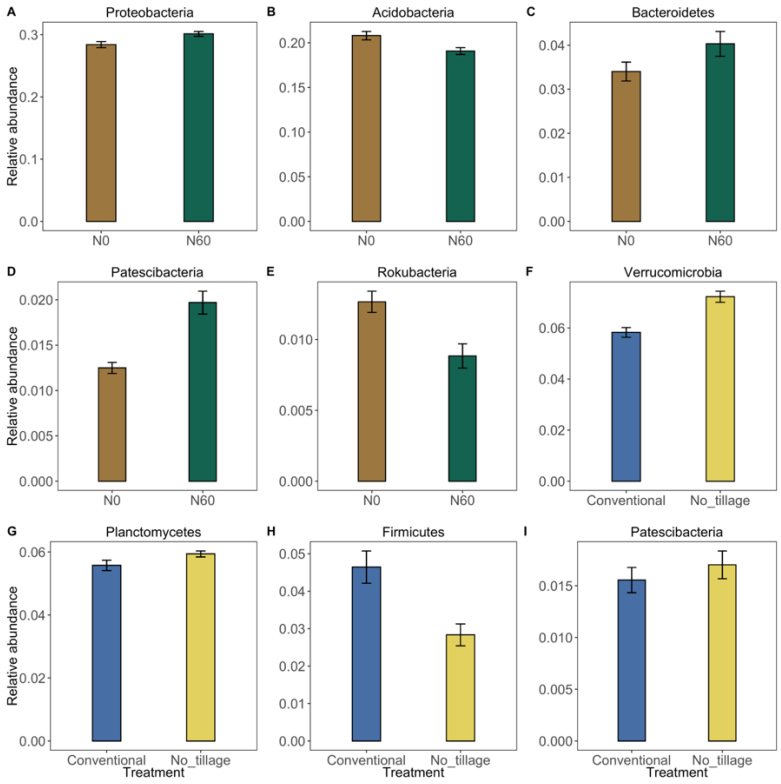


Figure 2.7 The relative abundance of dominant bacteria phyla (> 2% of total relative abundance) across fertilization and tillage management.

The significant influence of fertilization on *Protebacteria* (A), *Acidobacteria* (B), *Bacteroidetes* (C), *Patescibacteria* (D), *Rokubacteria* (E), *Verrucomicrobia* (F), *Planctomycetes* (G), *Firmicutes* (H) and *Patescibacteria* (I) based on results of Type III tests of fixed effects from Glimmix ANOVA generated by SAS system.

The relative abundance of *Proteobacteria* ($p < 0.05$), *Bacteroidetes* ($p < 0.05$), and *Patescibacteria* ($p < 0.0001$) (Fig. 1A, 1C, and 1D) were significantly greater in the N-fertilized treatments. Conversely, *Acidobacteria* ($p < 0.01$), and *Rokubacteria* ($p < 0.01$) were lower in N-fertilized plots (Fig. 1B and 1E). Moreover, tillage decreased the *Verrucomicrobia* ($p < 0.01$), *Planctomycetes* ($p < 0.05$), *Firmicutes* ($p < 0.01$), and *Patescibacteria* ($p < 0.05$) were lower in

the tilled soils but not *Firmicutes* ($p < 0.01$) (Fig. 1). No significant difference among these groups could be detected based on cover cropping or no cover cropping. The results indicated that the most abundant bacterial phyla tend to more sensitive to long-term fertilization and tillage rather than cover cropping management.

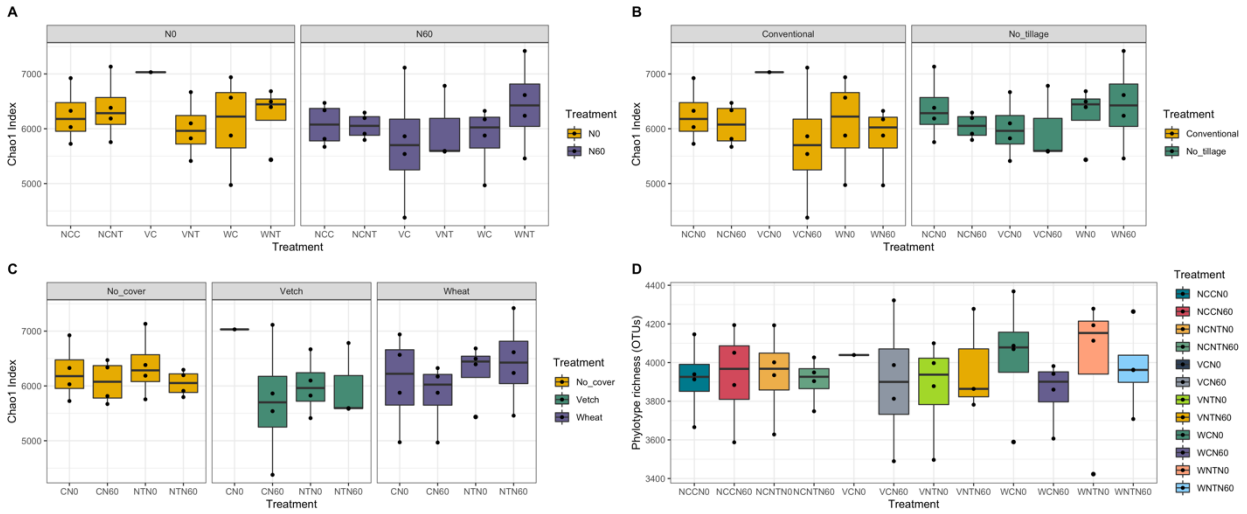


Figure 2.8 Alpha diversity of bacteria communities

The Chao1 in fertilization treatment (A); in tillage treatment (B); in cover cropping treatment (C). The phylotype richness (the number of OTUs) in 12 treatments (D). The boxplot bounds the interquartile range (IQR) divided by the median.

Table 2.2 The three-way PERMANOVA analysis of bacteria community in RCBD with five significant indicators (Fertilization, cover cropping, tillage, LAP, EOC)

	Df	Sum of Sqs	R^2	F. model	Pr(>F)	
Nitrogen	1	0.434	0.079	4.247	0.0001	***
Tillage	1	0.310	0.056	3.029	0.0017	**
Cover	2	0.350	0.064	1.714	0.023	*
AG	1	0.130	0.024	1.275	0.1896	
LAP	1	0.448	0.082	4.389	0.0002	***
EOC	1	0.183	0.033	1.786	0.0497	*
Residual	35	3.576	0.652			
Total	42	5.487	1.000			
Residual	33	3.086	0.643			
Adjusted R^2	0.218					

Significant codes: 0-0.001 '***', 0.001-0.01 '**', 0.01-0.05 '*'. The number of permutations was 9999.

Table 2.3 The three-way PERMANOVA analysis of viral community in RCBD with cover crops based upon results of RAPD-PCR

Source	Df	Sums of Sqs	Mean Sqs	F. Model	R^2	Pr (>F)	
Cover	2	0.907	0.454	1.244	0.055	0.047	*
Residuals	43	15.678	0.365		0.945		
Total	45	16.585			1.000		
Adjusted R2	0.011						

Table 2.4 The coding of sample ID

Sample_ID	Block	Treat	Cover	Nitrogen	Tillage
NCCN0_1	B1	NCCN0	No_cover	N0	Conventional
NCCN0_2	B2	NCCN0	No_cover	N0	Conventional
NCCN0_3	B3	NCCN0	No_cover	N0	Conventional
NCCN0_4	B4	NCCN0	No_cover	N0	Conventional
NCCN60_1	B1	NCCN60	No_cover	N60	Conventional
NCCN60_2	B2	NCCN60	No_cover	N60	Conventional
NCCN60_3	B3	NCCN60	No_cover	N60	Conventional
NCCN60_4	B4	NCCN60	No_cover	N60	Conventional
NCNTN0_1	B1	NCNTN0	No_cover	N0	No_tillage
NCNTN0_2	B2	NCNTN0	No_cover	N0	No_tillage
NCNTN0_3	B3	NCNTN0	No_cover	N0	No_tillage
NCNTN0_4	B4	NCNTN0	No_cover	N0	No_tillage
NCNTN60_1	B1	NCNTN60	No_cover	N60	No_tillage
NCNTN60_2	B2	NCNTN60	No_cover	N60	No_tillage
NCNTN60_3	B3	NCNTN60	No_cover	N60	No_tillage
NCNTN60_4	B4	NCNTN60	No_cover	N60	No_tillage
VCN60_1	B1	VCN60	Vetch	N60	Conventional
VCN60_2	B2	VCN60	Vetch	N60	Conventional
VCN60_3	B3	VCN60	Vetch	N60	Conventional
VCN60_4	B4	VCN60	Vetch	N60	Conventional
VNTN0_1	B1	VNTN0	Vetch	N0	No_tillage
VNTN0_2	B2	VNTN0	Vetch	N0	No_tillage
VNTN0_3	B3	VNTN0	Vetch	N0	No_tillage
VNTN0_4	B4	VNTN0	Vetch	N0	No_tillage
VNTN60_1	B1	VNTN60	Vetch	N60	No_tillage
VNTN60_2	B2	VNTN60	Vetch	N60	No_tillage
VNTN60_3	B3	VNTN60	Vetch	N60	No_tillage
VNTN60_4	B4	VNTN60	Vetch	N61	No_tillage
WCN0_1	B1	WCN0	Wheat	N0	Conventional
WCN0_2	B2	WCN0	Wheat	N0	Conventional
WCN0_3	B3	WCN0	Wheat	N0	Conventional
WCN0_4	B4	WCN0	Wheat	N0	Conventional
WCN60_1	B1	WCN60	Wheat	N60	Conventional
WCN60_2	B2	WCN60	Wheat	N60	Conventional
WCN60_3	B3	WCN60	Wheat	N60	Conventional
WCN60_4	B4	WCN60	Wheat	N60	Conventional
WNTN0_1	B1	WNTN0	Wheat	N0	No_tillage
WNTN0_2	B2	WNTN0	Wheat	N0	No_tillage
WNTN0_3	B3	WNTN0	Wheat	N0	No_tillage
WNTN0_4	B4	WNTN0	Wheat	N0	No_tillage
WNTN60_1	B1	WNTN60	Wheat	N60	No_tillage
WNTN60_2	B2	WNTN60	Wheat	N60	No_tillage
WNTN60_3	B3	WNTN60	Wheat	N60	No_tillage
WNTN60_4	B4	WNTN60	Wheat	N60	No_tillage

Chapter 3 Seasonal variation in bacterial community structure under long-term fertilization, tillage, and cover cropping

A version of this chapter is in preparation for submission to Frontier in Microbiology

by Ning Duan [a], Lidong Li [b], Xiaolong Liang [c], Aubrey Fine [a], Jie Zhuang [a, d], Mark Radosevich [a], Sean M. Schaeffer [a, *]

Contribution statement

Ning Duan carried out the experiment, molecular experiments, and all data processing and statistical analysis. Lidong helped to build a structural equation model. Xiaolong Liang helped with soil sampling, and Aubrey K. Fine measured the soil properties data. Jie Zhuang, Mark Radosevich, Sean M. Schaeffer are responsible for the project and help with the revision of the manuscript.

[a] Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

[b] USDA-Agriculture Research Service (ARS), Agroecosystem Management Research Unit, 255 Filley Hall, 3720 East Campus Loop South, Lincoln, NE 68583, United States

[c] Department of Earth and Planetary Sciences, Washington University in Saint Louis, Saint Louis, MO 63130, United States

d Center for Environmental Biotechnology, The University of Tennessee, Knoxville, TN 37996, United States

* Corresponding author. Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

Abstract

Soil bacterial communities are a critical control on ecosystem stability and function. Agricultural practices are known to alter microbial community structure and function. However, few studies have documented the seasonal variations of bacterial communities in soils under long-term conservation management practices. Here, we evaluated bacterial diversity with 12 combinations of fertilization, cover cropping and tillage throughout the cotton production cycle using 16S rRNA gene sequencing. Soil was collected from plots at the West Tennessee Agriculture Research and Education Center in Jackson, TN, which has been under continuous cotton production for more than 30 years. A total of 38,038 OTUs were detected across 171 samples. The dominant bacterial phyla were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Chloroflexi*, accounting for roughly 70% of the total bacterial community membership. Bacterial richness and evenness were maintained and even increased across the year. The effect of tillage was significant on the alpha diversity in soil samples collected in different seasons. The effect of fertilization and tillage on the structure of bacteria communities was continuous across four seasons ($p < 0.01$). However, cover cropping ($p < 0.05$) and soil moisture content ($p < 0.05$) only showed significant influence on the bacterial community structure after burn-down of cover crops and before planting of cotton, and was less apparent at peak cotton growth and after harvest. Nitrate-N appears to have a significant effect on the structure of bacteria communities after inorganic fertilization and at the peak of cotton growth ($p < 0.01$). Tillage and air temperature positively influenced denitrifying bacteria. Vetch had positive effects on nitrifying bacteria groups. Structural equation modeling revealed that N-fixing bacteria were sensitive to soil moisture content. From these results, we conclude that long-term tillage and fertilization are on-going factors increasing the diversity and

restructuring the composition bacterial communities, while cover cropping may have shorter-term effects on bacteria community structure. The bacterial taxa responding to management practices and environmental conditions were related to N fixation, nitrification, and denitrification. These findings suggest that long-term fertilization, tillage, and cover cropping established a steady system for maintaining bacterial diversity and strengthening the correlation between functional groups involved in N-cycling.

3.1 Introduction

Nitrogen (N) is a basic element for nucleic acids, proteins, etc. and is essential for soil organism growth and maintain their metabolic (Aczel, 2019). Microbes are the main drivers of soil N-cycling (Isobe & Ohte, 2014; Zhang et al., 2019a). Soil bacteria with different functions perform diverse ecological roles in agricultural systems (Aczel, 2019; Prasad et al., 2021). For example, N-cycling taxa related to N fixation, nitrification, and denitrification reflect the changes of N-processes and, which has been reported by many studies (Anand et al., 2015; Jiménez-Bueno et al., 2016; Liu et al., 2020). *Paenibacillus* strains with N-fixing capability have positive effect on weight of weight of crops and root growth (Liu et al., 2019). *Rhizobium* promoted the efficient for N fixation than grain legumes (Etemadi et al., 2018).

Long-term agricutural management tillage, inorganic N fertilization, and cropping system can influence bacteria diversity and functional groups (Rousk et al., 2009; Rousk et al., 2010a; Dai et al., 2018; Zhang et al., 2019a; Hu et al., 2021a). N fertilization significantly influences community structure (Ramirez et al., 2010b; Geisseler & Scow, 2014; Zhou et al., 2017).

Inorganic N fertilizer not only increases crop growth and increased soil organic matter content, but leads to increasing soil acidity, which decreased diversity of bacteria. N fertilization significantly can also impact ammonia oxidizing bacteria (AOB), For example, *Nitrosospira* were dominant in soils with N fertilizer (Bruns et al., 1999; Wu et al., 2011; Liu et al., 2018; Hu et al., 2021b)

Tillage management increases soil aeration and the degree of mixing of crop residues, decreases soil aggreagetion and water infiltration, thereby altering microenvironments and impacting microbial community structure through bottom up environmental controls (Kladivko, 2001; Lienhard et al., 2013). Bacterial diversitytends to decrease under conventional tillage, whereas reduced or no tillage can increase bacterial diversity due to acceleration of the decompositon rate of organic matter under conventional tillage (Wang et al., 2017; Legrand et al., 2018). However, no-tillage can drive a decrease of soil porosity and increase in bulk density leading to soil compaction compared to tillage (Logsdon & Karlen, 2004; Pastorelli et al., 2013), which will affect water and oxgen diffusion, and create anerobic environments favoring the growth of denitrifers (Tatti et al., 2015).

Cover cropping increases soil organic matter input, as well as protecting soil from erosion and nutrient loss through leaching and run-off (Romdhane et al., 2019). Also, soil surfaces covered by cover crop residue can reduce soil temperature fluctuation and water evaporation. Root exudates contain C-rich substrate, such as amino acids, organic acids, sugars, and phenolics, attracting a variety of rhizosphere microbes including N-fixing bacteria (Rasmann et al., 2005; Vukicevich et al., 2016b; Finney et al., 2017). Crop and root exudates positively influence soil

microbial biomass and activity (Bardgett & Shine, 1999; Bending et al., 2002; Mbuthia, 2014; Vukicevich et al., 2016a; Finney et al., 2017). The abundances of nitrifiers and nitrification processes can increase under the combination of crops and N fertilizer (Li et al., 2019a). Therefore, cover cropping has a significant influence on bacterial communities and functional taxa.

Studying the diversity of soil bacteria is important for estimation and prediction of soil function after a long-term agriculture management. In this study, we hypothesized that (1) bacterial diversity would be positively influenced by cover cropping and fertilization practices, and that conventional tillage would decrease bacterial diversity; (2) the bacterial community would be structured according to long-term soil management and vary with time throughout the cotton production cycle. (3) Tillage management would increase the abundances of denitrification bacteria. fertilization, and cover cropping would increase the abundance of bacteria functional taxa related to N-fixation, nitrification, and denitrification. We used 16S rRNA high-throughput amplicon sequencing to estimate bacterial diversity and relative abundance of various taxonomic groups.

3.2 Material and methods

3.2.1 Site description and sample collection

The long-term conservation management experimental site was established in 1981 and is a randomized complete block with split-split plot design. The site was located in Jackson,

Tennessee, USA (35°37′23.1″N 88°50′47.4″W) (Li et al., 2021). The soil is Lexington silt loam (fine-silty, mixed, thermic, Ultic Hapludalf). Inorganic nitrogen fertilizer (ammonium nitrate, NH_4NO_3) was applied at two levels of nitrogen (NH_4NO_3 at 0 and 67 kg ha^{-1}) as the main plots. Each main plot was divided into three subplots that contained three kinds of cover crop treatments (hairy vetch, *Vicia villosa* Roth; winter wheat, *Triticum aestivum* L. and no cover). Each subplot contained two tillage treatments (conventional tillage and no tillage). The tillage was performed before planting cotton using a standard disc harrow followed by smoothing and breaking up of clods by a triple-K harrow. There were 4 replications in each of 12 treatments (Fig. S1). The collected samples were coded with the following form: "sampling month", "type of cover crop" , "tillage or not", "fertilization or not" and "serial number of replicates", e.g. M_NCCN0_1 represents sampling in May from the treatment plot of no-cover, conventional tilled, no nitrogen fertilizer, and replicates 1; O_VNTN60_4 is sampled in October, vetch-covered, no-tilled with fertilization, replicates 4; D_WCN0_3 is sampled in December, wheat-covered, conventional tillage without fertilization, replicates 3.

The field sampling occurred four times during the cotton production in 2019. The first sampling was on May 21st, 2019, shortly after burn-down of the cover crop and just before planting in spring. The second sampling was on June 12th, 2019 after planting cotton but before fertilization in mid-summer. The third collection occurred on October 9th, 2019 at the peak of cotton growth in fall. The final sample collection was performed at the beginning of December just after harvest. Each sample was processed for 16S rRNA gene amplicon sequencing.

3.2.2 Pre-processing and DNA extraction

Soil samples were collected from 0 to 10 cm depth of four replicate plots in each of 12 treatments using a soil probe of 2.5 cm diameter. About 10-15 samples were randomly taken within each plot, 10-15 cm away from the center of crop row. The subsamples from each plot were thoroughly mixed into one composite sample and then placed into a zip-loc plastic bag. The samples were then transported in a cooler with ice bags within 24 hours to a lab freezer (-20 ℃). Small rocks and roots were removed from each soil sample before the soil was passed through 2-mm sieve.

Total soil DNA was extracted using DNeasy® PowerLyzer® Powersoil® kit (REF 12855, QIAGEN) from 0.25 g soil per sample following the manufacturer's instructions. Following extraction, the DNA was dissolved in sterile DNA-free PCR grade water. DNA quality and concentration were measured using NanoDrop (ND-3300 Fluorospectrometer 83060-50, Thermo Scientific).

3.2.3 Illumina MiSeq sequencing

DNeasy® PowerLyzer® Powersoil® kit (REF 12855, QIAGEN) was used to extract 48 soil samples, and the DNA was quantified using a Nanodrop (Thermo Scientific NanoDrop ND-3300 Fluorospectrometer 83060-50). The V3-V4 region of 16S rRNA genes was amplified using the published primers (341F 5'- CCTACGGGNGGCWGCAG-3'; 785R 5'-GACTACHVGGGTATCTAATCC-3') yielding a single amplicon of approximately 464 bp

(Klindworth et al., 2013). The PCR reaction (25 µL per sample) included 2.5 µL of microbial genomic DNA (5 ng / µL in 10 mM Tris pH 8.5), 5 µL each of forward and of reverse primer. Platinum Green Hot start master mix (12.5 µL of 2 ×) was used (Invitrogen™, Catalog No. 13001012, U.S.). PCR procedures involved in pre-denaturation at 95˚C for 5 minutes, followed by 25 cycles of denaturation at 95˚C for 40 seconds, annealing at 55˚C for 2 minutes, elongation at 72˚C for 1 minutes, and then held at 4˚C until stored in the freezer (-20 ˚C) for future use. Lastly, the samples were sequenced on an Illumina MiSeq platform with generation of 300 pair-end reads (Bartram et al., 2011) conducted at DNA Genomics Core, University of Tennessee. MiSeq Control Software version 2.6.2.1 was used in this study.

3.2.4 Sequence data analyses

Illumina sequencing data were examined by FastQC to perform the quality control (Andrews, 2010). Raw reads were processed using the Mothur pipeline (v. 1.35.1) (Schloss Laboratory; University of Michigan, Ann Arbor, MI). The analysis followed MiSeq SOP (https://mothur.org/wiki/miseq_sop/) (Kozich et al., 2013). The contigs with ambiguous bases were screened after the step of *make.contigs* and remaining high-quality contigs were aligned with SILVA bacterial reference database (v.132) using kmer searching. After de-noising the alignment and sequences, we used pre-cluster command split sequences by group and sorted them by abundance with no more than 1 difference per 100 bp of sequences. Chimeric sequences were removed using the VSERACH program (Rognes et al., 2016). Next, sequences were classified using the Ribosomal Database Project (RDP) Naive Bayesian classifier to reference sequences with cutoff of bootstrap value 80% (Wang et al., 2007). Sequences assigned as

Chloroplast, Mitochondria, unknown, Archaea, and Eukaryota were removed. Then sequences with no more than 3% difference were clustered into one OTU and taxonomic table was generated by *make.shared* command for further analysis (Schloss et al., 2009; Schloss, 2020). The sequences for each replicate were rarefied to 14,145 sequences to avoid the influence of sample size on diversity estimation. Results were analyzed using *phyloseq* (v1.30.0) and *vegan* (v 2.5.7) packages for diversity analyses and visualized in RStudio 1.1.463 interface. All the data has been deposited in in National Center for Biotechnology Information Databases (Sequence Read Archive), under BioProjects accession number is PRJNA747698.

3.2.5 Soil properties

Air temperature data were obtained from the National Weather Service Forecast Office. Soil pH was measured using a pH electrode (1 part soil-to-2 parts deionized water) (Ul-trabasic, Denver Instrument, Bohemia, NY, USA). Gravimetric water content (GWC; g water g^{-1} dry soil) was determined as mass loss of field moist soil after oven drying at 105 °C for 48 hours. Bulk soil was extracted using 0.5 M potassium sulfate (K_2SO_4) (10.0 g soil + 40 mL extractant) followed by vacuum filtration with 1.0 μm glass microfiber filters (Whatman GF/B). Briefly, two 10.0 g subsamples of soil from each composite plot sample were weighed into glass extraction vessels with one subsample receiving 1.0 mL ethanol-free chloroform to lyse microbial cells. Salt extracts were conducted with a potassium persulfate reagent overnight (80 °C) in sealed glass vials equipped with rubber septa for headspace gas analysis using an Infrared Gas Analyzer (IRGA, LI820, LiCor Inc., Lincoln, NE USA) (Doyle et al., 2004). Extracts were used for the

measurement of NO_3^-, and NH_4^+ concentration using microplate-based spectrophotometric determinations (Rhine et al., 1998; Doane & Horwáth, 2003)

3.2.6 Alpha-diversity and Beta-diversity measurement

Shannon-Wiener, the number of OTUs , and Pielou's evenness diversity indices indicate species richness and evenness (Kim et al., 2017). The Canonical analysis of principal coordinates analysis (CAP) was conducted to examine bacterial community structure between individual samples. Similarity percentage analysis (SIMPER) was also utilized to test percentage of dissimilarities for each species contribution at phylum level between two treatments (Grange & Smith, 2013).

3.2.7 Structural equation modeling

Structural equation modeling (AMOS 27; IBM Corporation, Meadville, PA, USA) was conducted to test the relationships between abundance of nitrifying, denitrifying, and N-fixing bacteria and effects of agricultural management. Path coefficients were tested by maximum likelihood estimation at $p \leq 0.05$. Multivariate normality was evaluated by Kurtosis value ≤ 7. Model fit was evaluated by (1) the minimum discrepancy divided by its degrees of freedom (CMIN/DF) in the range of 1 to 3 (Carmines & McIver, 1983), (2) the goodness of fit index (GFI) close to 1 (Tanaka & Huba, 1985), (3) the comparative fit index (CFI) close to 1 (Bentler, 1990), and (4) the root mean square error of approximation (RMSEA) less than 0.05 (Browne & Cudeck, 1993). We followed the procedures of developing and modifying a structural equation

model in Byrne (2013) and Li et al. (2019b). Briefly, we proposed an a priori model based on our hypotheses, tested if the existing pathways were significant and if necessary, pathways were left out, and then adjusted the priori model by dropping insignificant pathways and adding missing pathways with the consideration of model fit and scientific rationality.

3.2.8 Statistical analysis

We used Mixed Model Analysis of Variance to test the diversity indices (the number of OTUs, Shannon) across 11 treatments excluding the treatment of vetch covered, conventional tillage with no fertilization (VCN0). There were 3 out of 4 replicates in this treatment due to low quality scores (less than 20) poor assembly and alignment to the 16S rRNA reference sequences. Therefore, this treatment was removed from further analyses across all the sampling times. After the step of quality control, a total of 171 out of 192 samples were kept for the downstream analyses. Statistical analysis was conducted in SAS software with the Glimmix procedure (SAS Institute Inc., Cary, NC), the normality, equal variance and outliers were checked using UNIVARIATE procedure. The least square means compared with Fisher's Least Significant Difference (LSD) at 5% significance level and Tukey's Honest Significant Difference (Tukey's HSD) tests were performed to compare differences between each treatment. The field plot map is shown in Fig. S1. The randomized complete block design with split-split plot model was used: $Y_{ijkl}=\mu+B_i+F_j+B*F_{ij}+C_k+C*F_{jk}+B*C*F_{ijk}+T_l+T*F_{jl}+T*C_{kl}+T*C*F_{jkl}+B*\ T*C*F_{ijkl}$ (Y is the response variable, μ is residuals, B is block regarded as random effects, F is fertilization, C is cover crops, and T is tillage treatment, three treatments are fixed effect). The tillage, fertilization, cover cropping, and their interaction effects had been tested in this study. The statistical test on

seasonal variation with regard to seasonal sampling as repeated measures based on RCBD split-split plot design. Furthermore, ANOVA with permutation test was also conducted in R to estimate if a given treatment signal was significant or not (Anderson, 2014).

Spearman's correlation was performed among bacteria, at the genus level, involved in N-fixation, nitrification, and denitrification in each sampling seasons, significant correlations ($p < 0.05$) were correlated using "BH" method (Benjamini, 1995). All functional taxa mentioned in this study were by genus found in our samples against previous studies (Pagan et al., 1975; Pichinoty et al., 1976; Carlson & Ingraham, 1983; Davey & Marchant, 1983; Awonaike et al., 1990; Gee et al., 1990; Urakami et al., 1995; Baumann et al., 1996; Albrecht et al., 1997; Schramm et al., 1999; Hurek & Reinhold-Hurek, 2003; Verbaendert et al., 2011; Kostka et al., 2012; Cua & Stein, 2014; Sun et al., 2016; Fukami et al., 2018; Cardoso & Kana, 2019; Liu et al., 2019; Zhang et al., 2019b; Spieck et al., 2020).

3.3 Results

In total, 4,854,968; 5,495,390; 3,983,387; and 3,809,793 raw sequence reads were obtained from 44 out of 48 samples collected in May, June, October, and December, respectively. The remaining four samples of each sampling season from various treatment plots (M_VCN0_1, M_VCN0_2, M_VCN0_3 , M_VNTN60_4, J_VCN0_1, J_VCN0_2, J_VCN0_3, J_VNTN60_4, O_VCN0_1, O_VCN0_2, O_VCN0_3, O_VNTN60_4, D_VCN0_1, D_VCN0_2, D_VCN0_3, D_NCCN0_4, and D_VNTN60_4) were filtered out and eliminated from further analyses using the Mothur pipeline due to low quality scores (less than 20) poor assembly and alignment to the

16S rRNA reference sequences. A total of 38,038 OTUs with 97% identity were generated from 171 samples after rarefaction. The number of OTUs per sample ranged from 4134 (D_VCN60_1) to 2758 (O_NCNTN0_4).

3.2.1 Contribution of bacterial phyla and bacterial species involved in nitrogen cycling

Proteobacteria, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Chloroflexi* were the most abundant bacterial phyla in this study (Fig. S3.2). SIMPER analysis (analysis of similarity of percentage) was used to estimate the contribution of each bacterial phyla and calculate the contribution of each bacterial taxonomic group to the dissimilarity of the whole bacterial community between different treatments (Warton et al., 2012). All phyla contributing more than 2% were included (Fig. 3.1). The largest contribution to the difference between no cover and vetch, no cover and wheat, vetch and wheat, tillage and no tillage, fertilization and no fertilization were attributed to *Firmicutes*, *Cyanobacteria* and *Actinobacteria*. All phyla showed a similar variation of contribution in five pair-wise comparisons across seasons. The contribution of *Firmicutes*, *Actinobacteria*, *Patescibacteria* and *Nitrospirae* increased after planting cotton but before fertilization, decreased at the peak of cotton growth and increased again after harvest. However, the *Cyanobacteria*, *Chloroflexi*, *Acidobacteria,* and *Planctomycetes* exhibited the opposite trend (Fig. 3.1).

3.3.2 Seasonal Variations of soil bacteria diversity under long-term managements

The diversity, richness, and evenness of the bacterial communities in different samples were estimated by Shannon index (H), the number of OTUs, and Pielou's index, respectively (Fig. 3.2). No significant interaction effect among tillage, fertilization, cover cropping, and seasons was observed. Only season and tillage management had a significant influence on bacterial richness and evenness (Table S1). Shannon index ($p < 0.001$), number of OTUs ($p < 0.01$), and Pielou's evenness ($p < 0.01$) significantly varied across seasons (Fig. 3.1A to C). Samples collected in June contained greater diversity than May which might be due to greater richness ($p < 0.05$). but soils collected in December and October had significantly greater richness and evenness than diversity estimated in May ($p < 0.05$). Soils sampled December had greater diversity than October because of higher richness ($p < 0.05$). However, bacterial diversity has no different between June and October. In addition, bacterial Shannon index ($p < 0.01$), numbers of OTUs ($p < 0.01$), amd Pielou's evenness ($p < 0.05$) increased relatively more in conventional tillage than in no tillage (Fig. 3.2D to E). The results indicate that bacterial diversity was sensitive to the tillage management than fertilization and cover cropping, and environmental factors might be responsible for variation of bacterial diversity across seasons.

3.3.3 Comparisons of bacterial community structure across long-term tillage, fertilization, and cover cropping

Canonical analysis of principal coordinates analysis (CAP) and analysis of variance (ANOVA) with permutation on ordination were performed to estimate importance of environmental

variables and three managements on shaping bacterial community structure according to sampling time in the cotton production cycle. Overall, spatial variation of bacterial communities was significantly influenced by fertilization and tillage across four sampling seasons. Cover cropping and soil water content were significant predictors explaining the variation in bacterial communities of samples collected after burn-down cover crops (Fig. 3.3A). Bacterial communities were significant influenced by concentration of nitrate in the samples collected after fertilization and at the peak of cotton growth (Fig. 3.3C). About 7.1% to 15.2% of the variation of bacterial communities can be explained by the environment variables within the two CAP axes across May, June, October, and December (Fig. 3.3, Table S3.2). The results suggest that fertilization and tillage have a long-lasting impact on bacterial communities, and cover cropping only exhibits the short-term effect after practices of burn-down of cover crops.

3.3.4 Changes of bacterial genus involve in N fixation, nitrification, and denitrification across long-term management and seasonal environmental change

Based on literature searching, N fixing bacteria, such as *Azoarcus*, *Paenibacillus*, *Rhizobium*, *Azospirillum*, *Nostoc Bradyrhizobium*, and Nitrifying bacteria including *Nitrosospira*, *Nitrosomonas*, *Nitrolancea*, and bacteria for denitrification, *Flavobacterium*, *Streptomyces*, *Bacillus*, *Pseudomonas*, *Paracoccus*, *Cupriavidus*, *Sphingomonas*, *Mycobacterium*, *Rhodococcus*, *Rhodanobacter*, and *Hyphomicrobium* can be retrieved in our soil samples. Overall, bacterial genera involved in N fixation, nitrification, and denitrification were more connected in more warmer seasons (May and June) than cold season (October and December). After burn-down cover crops, approximately about 9.10 % of correlation was found between N

fixing bacteria and nitrifying bacteria, and 45.45% of connections between N fixing bacteria and denitrification bacteria (Fig. 3.5A). After tillage and before fertilization, there were about 6.67% and 46. 67% connection between N fixing bacteria, nitrifying, and denitrifying bacteria, respectively. Positive correlation between nitrifying and denitrifying bacteria took 26.67 % (Fig. 3.5B). After fertilization and cotton harvest, the most of positive connections were observed between N fixing and denitrifying bacteria (Figs. 3.5C and 3.5D).

As shown in the structural equation model for estimating seasonal influence of tillage, fertilization, and cover cropping on N-fixing, nitrifying, and denitrifying bacteria abundances, abundances of N-fixing bacteria have positive effect on nitrifying (standardized path coefficient = 0.28, $p < 0.001$) and denitrifying bacteria (standardized path coefficient = 0.54, $p < 0.001$), respectively. As for effect of cover crop, Vetch covered soil can increase abundance of nitrifying bacteria (standardized path coefficient = 0.28, $p < 0.001$). Tillage increased abundances of denitrifying bacteria (standardized path coefficient = 0.27, $p < 0.001$). N-fixing bacteria were negatively influenced by moisture content (standardized path coefficient = 0.27, $p < 0.001$) and temperature (standardized path coefficient = 0.27, $p < 0.001$) (Table S3.3).

3.4 Discussion

3.4.1 Bacterial diversity and communities structure influenced by the agricultural management across the four seasons.

The goal of the study was to evaluate the variation of bacterial community composition under long-term (40+ years) conservation management systems and seasonal patterns during the cotton production cycle in West Tennessee. The amplicon-based metagenomic sequencing of the bacterial 16S rRNA V3-V4 variable region indicates that bacterial alpha diversity (Shannon and number of OTUs) increased twice across four sampling seasons. The first time of alpha diversity increased occurred after tillage, the second increase occurred between the stage of the peak of cotton growth and after harvest. We found that tillage had a greater effect on increasing bacterial community richness and evenness. Meanwhile, tillage and fertilization together had an effect on bacterial community structure lasting across a whole year. Tillage management disturbs the stability of soil aggregates and releases protected organic matter (Rong et al., 2017; Bu et al., 2020). Soil aeration porosity and oxygen diffusion rate can be improved by tillage, providing suitable environment for aerobic microbial growth and accelerating the decomposition of soil organic matter as substrate and energy source for soil microbes (Linn & Doran, 1984a; Khan, 1996; Simmons & Coleman, 2008). In addition, different microbial functional groups are harbored at different soil depths, and tillage practices might relocate microbial groups in the deeper soil to the soil surface leading to increase of bacterial richness and evenness and structural of bacterial communities. In contrast, during rainfall events, the transport of bacteria from surface soils might be much more significant in the no-till soil than in the tilled soil (Chen

et al., 2021). This no-till enhanced mobility of bacteria might influence soil bacterial community composition.

In respect to the influence of inorganic N fertilization on bacterial communities, microbial biomass, bacteria community structure, and specific taxonomic responses to inorganic fertilization management have been reported in many studies (Ramirez et al., 2010c; Geisseler & Scow, 2014; Zhou et al., 2017). We observed an effect of cover cropping on bacterial structure after burn-down of cover crops. In this agroecosystem, all the cover crop residues are left on the soil surface, which increases soil organic C and N availability (in the case of N-fixing crops), and can strongly facilitate increasing of bacterial abundances and diversity (Vukicevich et al., 2016b; Schmidt et al., 2018). Meanwhile, soil with crop residue may show improved soil moisture content, which may also shape bacterial communities (Acharya et al., 2019). We observed that Nitrate-N showed a significant influence on bacteria communities in October, probably due to an increase in nitrate-N from the application of inorganic N fertilizer (ammonium nitrate in this study), which would influence N-fixing bacteria, nitrifier and/or denitrifier populations (Ouyang et al., 2018; Norton & Ouyang, 2019).

Furthermore, compared to fertilization and tillage, cover crops appear to have a short-term effect on structure of bacterial communities. In this study, cover crops were planted and grown in winter and spring seasons, and the cover crops residues were left on the soil surface. The nutrient composition of wheat and vetch are quite different. Winter wheat possesses high C/N ratio compared to hairy vetch, which affects the quality and quantity of carbon, nitrogen, and other nutrient resources for bacteria. Plant residues with different nutrient composition could

significantly reshape the soil bacterial community structure (Marschner et al., 2003). For example, soil enriched with relatively more chemically recalcitrant substrates often exhibits enrichment in microbes with the ability to secrete extracellular enzymes to degrade complex polymers (e.g. cellulase) (Marschner et al., 2003). Our results indicate that the lasting effect of field managements over three decades is mainly reflected in tillage and fertilization treatments, which has also been previously reported (Toda & Uchida, 2017).

3.4.2 Bacterial taxa involving in N-cycling under tillage, fertilization, and cover cropping across seasons.

As a conceptual model, the "N saturation hypothesis", stresses that when N supply exceeds biological demand, there is a resulting loss of N from the ecosystem (Aber et al., 1998; Galloway et al., 2003; Pajares & Bohannan, 2016). Here, we observed that N-fixing cover crops have a substantial positive effect on denitrifying bacterial groups across four sampling seasons, which may provide the evidence that N in this long-term soil system may meet, or exceed, the N needs of bacteria. N fixation and denitrification process driven by bacteria may be coupled and lead to maintenance of the balance between biological N input and loss pathways in long-term tillage, fertilization, and cover cropping treated systems.

Nitrification process driven by nitrifiers responded to crop residues with low C:N ratio input. Low C:N ratio of vetch compared to that of other cover crops provides more readily utilizable substrate for bacterial decomposition. N input by N-fixing cover crops can also increase the

activity and abundance of nitrifiers that use it for metabolic function, either as an oxidizable energy source, or terminal electron receptor (Songjuan et al., 2021).

Conventional tillage disrupts the soil aggregate structure, which affects denitrifiers (Dick, 1992; Buckley & Schmidt, 2001; Kladivko, 2001; Lienhard et al., 2013). In our study, tillage showed a positive effect on denitrifier abundance, which agrees with studies showing greater denitrification gene abundance under similar conditions (Wang et al., 2019). This may be due to either denitrifiers from deeper soil being brought to the soil surface by tillage, or tillage practices that compact the soil the spaces between soil particles and reduce oxygen diffusion (Holland, 2004; Wang et al., 2017).

As for the moisture content negatively influencing the abundance of N-fixing bacteria, some free-living N-fixing bacteria can survive from drought stress (Fuhrmann et al., 1986). The survival and distribution of N-fixing bacteria are not necessarily restricted by anaerobic conditions created by higher soil moisture, but can be influenced by the initial moisture content and moisture stress in water-filled pores of soil microhabitats (Postma et al., 1989; Zahran, 1999). The balance between growth in aerobic conditions and undesirable conditions for survival and unevenly distributed microhabitat created by moisture stress may contribute to the how N-fixing bacteria response to soil moisture, which needs to be further investigated.

3.5 Conclusions

Overall, we have shown that bacterial communities were influenced by long-term agricultural management practices and seasonal climate variation. Analysis of bacterial community structure with 16S rRNA amplicon-based metagenomics sequencing suggests that fertilization and tillage had a stronger and more lasting effect on the structure of bacterial communities relative to tillage and cover cropping management. Alpha diversity of the bacterial community was only influenced by tillage. Cover cropping had a short-term effect on the structure of bacteria communities and occurred after burning down all cover crops. Bacterial taxa known to be associated with N fixation, nitrification, and denitrification were directly and strongly influenced by tillage, vetch cover, and air temperature, and soil moisture content. In conclusion, long-term conventional tillage, fertilization, and cover cropping not dampen the diversity of bacteria communities. These management practices promoted the connection between N-cycling functional groups.

References

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I. 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. BioScience, 48(11), 921-934.

Acharya, B. S., Dodla, S., Gaston, L. A., Darapuneni, M., Wang, J. J., Sepat, S., Bohara, H. 2019. Winter cover crops effect on soil moisture and soybean growth and yield under different tillage systems. Soil and Tillage Research, 195, 104430.

Aczel, M. R. 2019. What is the nitrogen cycle and why is it key to life?

Albrecht, A., Ottow, J., Benckiser, G., Sich, I., Russow, R. 1997. Incomplete denitrification (NO and N2O) from nitrate by Streptomyces violaceoruber and S. nitrosporeus revealed by acetylene inhibition and 15N gas chromatography-quadrupole mass spectrometry analyses. Naturwissenschaften, 84(4), 145-147.

Anand, M., Baidyanath, K., Dina, N. 2015. Cyanobacterial consortium in the improvement of maize crop. International Journal of Current Microbiology and Applied Sciences, 4(3), 264-274.

Anderson, M. J. 2014. Permutational multivariate analysis of variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online, 1-15.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data.

Awonaike, K., Kumarasinghe, K., Danso, S. 1990. Nitrogen fixation and yield of cowpea (Vigna unguiculata) as influenced by cultivar and Bradyrhizobium strain. Field Crops Research, 24(3-4), 163-171.

Bardgett, R. D., Shine, A. 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology & Biochemistry, 31(2), 317-321.

Baumann, B., Snozzi, M., Zehnder, A., Van Der Meer, J. R. 1996. Dynamics of denitrification activity of Paracoccus denitrificans in continuous culture during aerobic-anaerobic changes. Journal of bacteriology, 178(15), 4367-4374.

Bending, G. D., Turner, M. K., Jones, J. E. J. S. B., Biochemistry. 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. 34(8), 1073-1082.

Benjamini, Y. 1995. Benjamini and Hochberg methods. Journal of the Royal statistical society: series B (Methodological), 57, 289-300.

Bentler, P. M. 1990. Comparative fit indexes in structural models. Psychological bulletin, 107(2), 238.

Browne, M. W., Cudeck, R. 1993. Alternative ways of assessing model fit In: Bollen KA, Long JS, editors. Testing structural equation models. Beverly Hills, CA: Sage, 111-135.

Bruns, M. A., Stephen, J. R., Kowalchuk, G. A., Prosser, J. I., Paul, E. A. 1999. Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled, and successional soils. Applied and environmental microbiology, 65(7), 2994-3000.

Bu, R., Ren, T., Lei, M., Liu, B., Li, X., Cong, R., Zhang, Y., Lu, J. 2020. Tillage and straw-returning practices effect on soil dissolved organic matter, aggregate fraction and bacteria community under rice-rice-rapeseed rotation system. Agriculture, Ecosystems & Environment, 287, 106681.

Buckley, D., Schmidt, T. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microbial Ecology, 42(1), 11-21.

Byrne, B. M. 2013. Structural equation modeling with AMOS: Basic concepts, applications, and programming (2nd ed.): New York: Routledge.

Cardoso, N. C., Kana, B. D. 2019. The contribution of the NarB and NarGHI enzymes to nitrate reduction in Mycobacterium smegmatis.

Carlson, C. A., Ingraham, J. L. 1983. Comparison of denitrification by Pseudomonas stutzeri, Pseudomonas aeruginosa, and Paracoccus denitrificans. Applied and environmental microbiology, 45(4), 1247-1253.

Carmines, E. G., McIver, J. P. 1983. An introduction to the analysis of models with unobserved variables. Political Methodology, 51-102.

Cua, L. S., Stein, L. Y. 2014. Characterization of denitrifying activity by the alphaproteobacterium, Sphingomonas wittichii RW1. Frontiers in Microbiology, 5, 404.

Dai, Z., Su, W., Chen, H., Barberán, A., Zhao, H., Yu, M., Yu, L., Brookes, P. C., Schadt, C. W., Chang, S. X. 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. Global Change Biology, 24(8), 3452-3461.

Davey, A., Marchant, H. J. 1983. Seasonal variation in nitrogen fixation by Nostoc commune Vaucher at the Vestfold Hills, Antarctica. Phycologia, 22(4), 377-385.

Dawson, R., Murphy, K. 1972. The temperature dependency of biological denitrification. Water Research, 6(1), 71-83.

Dick, R. P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agriculture, Ecosystems & Environment, 40(1-4), 25-36.

Doane, T. A., Horwáth, W. R. 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical letters, 36(12), 2713-2722.

Etemadi, F., Hashemi, M., Zandvakili, O., Dolatabadian, A., Sadeghpour, A. 2018. Nitrogen contribution from winter-killed faba bean cover crop to spring-sown sweet corn in conventional and no-till systems. Agronomy Journal, 110(2), 455-462.

Finney, D. M., Buyer, J. S., Kaye, J. P. 2017. Living cover crops have immediate impacts on soil microbial community structure and function. Journal of Soil and Water Conservation, 72(4), 361-373.

Fuhrmann, J., Davey, C., Wollum, A. 1986. Desiccation tolerance of clover rhizobia in sterile soils. Soil Science Society of America Journal, 50(3), 639-644.

Fukami, J., Cerezini, P., Hungria, M. 2018. Azospirillum: benefits that go far beyond biological nitrogen fixation. AMB Express, 8(1), 1-12.

Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B., Cosby, B. J. 2003. The nitrogen cascade. BioScience, 53(4), 341-356.

Gee, C. S., Pfeffer, J. T., Suidan, M. T. 1990. Nitrosomonas and Nitrobacter interactions in biological nitrification. Journal of environmental Engineering, 116(1), 4-17.

Geisseler, D., Scow, K. M. 2014. Long-term effects of mineral fertilizers on soil microorganisms–A review. Soil Biology and Biochemistry, 75, 54-63.

Grange, L. J., Smith, C. R. 2013. Megafaunal communities in rapidly warming fjords along the West Antarctic Peninsula: hotspots of abundance and beta diversity. PLoS One, 8(12), e77917.

Holland, J. M. 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agriculture, Ecosystems & Environment, 103(1), 1-25.

Hurek, T., Reinhold-Hurek, B. 2003. Azoarcus sp. strain BH72 as a model for nitrogen-fixing grass endophytes. Journal of biotechnology, 106(2-3), 169-178.

Isobe, K., Ohte, N. 2014. Ecological perspectives on microbes involved in N-cycling. Microbes and environments, ME13159.

Jiménez-Bueno, N., Valenzuela-Encinas, C., Marsch, R., Ortiz-Gutiérrez, D., Verhulst, N., Govaerts, B., Dendooven, L., Navarro-Noya, Y. 2016. Bacterial indicator taxa in soils under different long-term agricultural management. Journal of applied microbiology, 120(4), 921-933.

Khan, A. 1996. Influence of tillage on soil aeration. Journal of agronomy and crop science, 177(4), 253-259.

Kim, B. R., Shin, J., Guevarra, R. B., Lee, J. H., Kim, D. W., Seol, K. H., Lee, J. H., Kim, H. B., Isaacson, R. E. 2017. Deciphering Diversity Indices for a Better Understanding of Microbial Communities. Journal of Microbiology and Biotechnology, 27(12), 2089-2093.

Kim, N., Riggins, C., Rodriguez-Zas, S., Zabaloy, M. C., Villamil, M. B. 2021. Long-term residue removal under tillage decreases amoA-nitrifiers and stimulates nirS-denitrifier groups in the soil. Applied Soil Ecology, 157, 103730.

Kladivko, E. J. 2001. Tillage systems and soil ecology. Soil & Tillage Research, 61(1-2), 61-76.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F. O. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic acids research, 41(1), e1-e1.

Kostka, J. E., Green, S. J., Rishishwar, L., Prakash, O., Katz, L. S., Mariño-Ramírez, L., Jordan, I. K., Munk, C., Ivanova, N., Mikhailova, N. (2012). Genome sequences for six

Rhodanobacter strains, isolated from soils and the terrestrial subsurface, with variable denitrification capabilities. In: Am Soc Microbiol.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., Schloss, P. D. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology, 79(17), 5112-5120.

Legrand, F., Picot, A., Cobo-Díaz, J. F., Carof, M., Chen, W., Le Floch, G. 2018. Effect of tillage and static abiotic soil properties on microbial diversity. Applied Soil Ecology, 132, 135-145.

Li, H., Zhang, Y., Yang, S., Wang, Z., Feng, X., Liu, H., Jiang, Y. 2019a. Variations in soil bacterial taxonomic profiles and putative functions in response to straw incorporation combined with N fertilization during the maize growing season. Agriculture, Ecosystems & Environment, 283, 106578.

Li, L., Konkel, J., Jin, V. L., Schaeffer, S. M. 2021. Conservation management improves agroecosystem function and resilience of soil nitrogen cycling in response to seasonal changes in climate. Science of the total environment, 779, 146457.

Li, L., Wilson, C. B., He, H., Zhang, X., Zhou, F., Schaeffer, S. M. 2019b. Physical, biochemical, and microbial controls on amino sugar accumulation in soils under long-term cover cropping and no-tillage farming. Soil Biology and Biochemistry, 135, 369-378.

Lienhard, P., Tivet, F., Chabanne, A., Dequiedt, S., Lelièvre, M., Sayphoummie, S., Leudphanane, B., Prévost-Bouré, N. C., Séguy, L., Maron, P.-A. J. A. f. s. d. 2013. No-

till and cover crops shift soil microbial abundance and diversity in Laos tropical grasslands. 33(2), 375-384.

Linn, D., Doran, J. J. S. S. S. o. A. J. 1984. Aerobic and Anaerobic Microbial Populations in No-till and Plowed Soils 48(4), 794-799.

Liu, S., Coyne, M., Grove, J., Flythe, M. 2018. Nitrogen, season, and tillage management influence ammonia oxidizing bacterial communities in long-term maize. Applied Soil Ecology, 129, 98-106.

Liu, X., Li, Q., Li, Y., Guan, G., Chen, S. 2019. Paenibacillus strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. PeerJ, 7, e7445.

Liu, X., Li, Y., Ren, X., Chen, B., Zhang, Y., Shen, C., Wang, F., Wu, D. 2020. Long-term greenhouse cucumber production alters soil bacterial community structure. Journal of Soil Science and Plant Nutrition, 20(2), 306-321.

Logsdon, S. D., Karlen, D. L. 2004. Bulk density as a soil quality indicator during conversion to no-tillage. Soil and Tillage Research, 78(2), 143-149.

Marschner, P., Kandeler, E., Marschner, B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry, 35(3), 453-461.

Mbuthia, L. W. 2014. Long-Term Impacts of Tillage, Cover Crops, and Nitrogen Rates on Microbial Community Dynamics and Soil Quality Parameters under Continuous Cotton Production in West Tennessee.

Nevins, C. J., Nakatsu, C., Armstrong, S. 2018. Characterization of microbial community response to cover crop residue decomposition. Soil Biology and Biochemistry, 127, 39-49.

Norton, J., Ouyang, Y. 2019. Controls and adaptive management of nitrification in agricultural soils. Frontiers in Microbiology, 10, 1931.

Ouyang, Y., Evans, S. E., Friesen, M. L., Tiemann, L. K. 2018. Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: a meta-analysis of field studies. Soil Biology and Biochemistry, 127, 71-78.

Pagan, J., Child, J., Scowcroft, W., Gibson, A. 1975. Nitrogen fixation by Rhizobium cultured on a defined medium. Nature, 256(5516), 406-407.

Pajares, S., Bohannan, B. J. 2016. Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. Frontiers in Microbiology, 7, 1045.

Pastorelli, R., Vignozzi, N., Landi, S., Piccolo, R., Orsini, R., Seddaiu, G., Roggero, P. P., Pagliai, M. 2013. Consequences on macroporosity and bacterial diversity of adopting a no-tillage farming system in a clayish soil of Central Italy. Soil Biology and Biochemistry, 66, 78-93.

Pichinoty, F., Bigliardi-Rouvier, J., Mandel, M., Greenway, B., Méténier, G., Garcia, J.-L. 1976. The isolation and properties of a denitrifying bacterium of the genus Flavobacterium. Antonie Van Leeuwenhoek, 42(3), 349-354.

Postma, J., Van Veen, J., Walter, S. 1989. Influence of different initial soil moisture contents on the distribution and population dynamics of introduced Rhizobium leguminosarum biovar trifolii. Soil Biology and Biochemistry, 21(3), 437-442.

Prasad, S., Malav, L. C., Choudhary, J., Kannojiya, S., Kundu, M., Kumar, S., Yadav, A. N. (2021). Soil microbiomes for healthy nutrient recycling. In *Current Trends in Microbial Biotechnology for Sustainable Agriculture* (pp. 1-21): Springer.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., Fierer, N. 2010a. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology, 91(12), 3463-3470.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., Fierer, N. J. E. 2010b. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. 91(12), 3463-3470.

Rasmann, S., Kollner, T. G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., Turlings, T. C. J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature, 434(7034), 732-737.

Rhine, E. D., Mulvaney, R. L., Pratt, E. J., Sims, G. K. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Science Society of America Journal, 62(2), 473-480.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F. J. P. 2016. VSEARCH: a versatile open source tool for metagenomics. 4, e2584.

Romdhane, S., Spor, A., Busset, H., Falchetto, L., Martin, J., Bizouard, F., Bru, D., Breuil, M. C., Philippot, L., Cordeau, S. 2019. Cover Crop Management Practices Rather Than Composition of Cover Crop Mixtures Affect Bacterial Communities in No-Till Agroecosystems. Frontiers in Microbiology, 10.

Rong, Q., Cai, Y., Chen, B., Yue, W., Tan, Q. 2017. An enhanced export coefficient based optimization model for supporting agricultural nonpoint source pollution mitigation under uncertainty. Science of the total environment, 580, 1351-1362.

Rousk, J., Baath, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., Fierer, N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J, 4(10), 1340-1351.

Rousk, J., Brookes, P. C., Baath, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol, 75(6), 1589-1596.

Schloss, P. D. 2020. Reintroducing mothur: 10 years later. Applied and environmental microbiology, 86(2).

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and environmental microbiology, 75(23), 7537-7541.

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J., Scow, K. 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLoS One, 13(2), e0192953.

Schramm, A., de Beer, D., van den Heuvel, J. C., Ottengraf, S., Amann, R. 1999. Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Applied and environmental microbiology, 65(8), 3690-3696.

Simmons, B. L., Coleman, D. C. J. A. s. e. 2008. Microbial community response to transition from conventional to conservation tillage in cotton fields. 40(3), 518-528.

Songjuan, G., Weidong, C., Guopeng, Z. 2021. Bacterial communities in paddy soils changed by milk vetch as green manure: A study conducted across six provinces in South China. Pedosphere, 31(4), 521-530.

Spieck, E., Sass, K., Keuter, S., Hirschmann, S., Spohn, M., Indenbirken, D., Kop, L. F., Lücker, S., Giaveno, A. 2020. Defining culture conditions for the hidden nitrite-oxidizing bacterium Nitrolancea. Frontiers in Microbiology, 11, 1522.

Sun, Z., Lv, Y., Liu, Y., Ren, R. 2016. Removal of nitrogen by heterotrophic nitrification-aerobic denitrification of a novel metal resistant bacterium Cupriavidus sp. S1. Bioresource technology, 220, 142-150.

Tanaka, J. S., Huba, G. J. 1985. A fit index for covariance structure models under arbitrary GLS estimation. British Journal of Mathematical and Statistical Psychology, 38(2), 197-201.

Tatti, E., Goyer, C., Burton, D. L., Wertz, S., Zebarth, B. J., Chantigny, M., Filion, M. 2015. Tillage management and seasonal effects on denitrifier community abundance, gene expression and structure over winter. Microbial Ecology, 70(3), 795-808.

Toda, M., Uchida, Y. 2017. Long-term use of green manure legume and chemical fertiliser affect soil bacterial community structures but not the rate of soil nitrate decrease when excess carbon and nitrogen are applied. Soil Research, 55(6), 524-533.

Urakami, T., Sasaki, J., Suzuki, K.-I., Komagata, K. 1995. Characterization and Description of Hyphomicrobium denitrificans sp. nov. International Journal of Systematic and Evolutionary Microbiology, 45(3), 528-532.

Verbaendert, I., Boon, N., De Vos, P., Heylen, K. 2011. Denitrification is a common feature among members of the genus Bacillus. Systematic and Applied Microbiology, 34(5), 385-391.

Vukicevich, E., Lowery, T., Bowen, P., Urbez-Torres, J. R., Hart, M. 2016a. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development, 36(3).

Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J. R., Hart, M. 2016b. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development, 36(3), 1-14.

Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and environmental microbiology, 73(16), 5261-5267.

Wang, W., Yang, M., Shen, P., Zhang, R., Qin, X., Han, J., Li, Y., Wen, X., Liao, Y. 2019. Conservation tillage reduces nitrous oxide emissions by regulating functional genes for ammonia oxidation and denitrification in a winter wheat ecosystem. Soil and Tillage Research, 194, 104347.

Wang, Y., Li, C., Tu, C., Hoyt, G. D., DeForest, J. L., Hu, S. 2017. Long-term no-tillage and organic input management enhanced the diversity and stability of soil microbial community. Science of the total environment, 609, 341-347.

Warton, D. I., Wright, S. T., Wang, Y. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution, 3(1), 89-101.

Wu, Y., Lu, L., Wang, B., Lin, X., Zhu, J., Cai, Z., Yan, X., Jia, Z. 2011. Long-term field fertilization significantly alters community structure of ammonia-oxidizing bacteria rather than archaea in a paddy soil. Soil Science Society of America Journal, 75(4), 1431-1439.

Zahran, H. H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and molecular biology reviews, 63(4), 968-989.

Zhan, M.-j., Xia, L., Zhan, L., Wang, Y. 2019. Recognition of changes in air and soil temperatures at a station typical of China's subtropical monsoon region (1961–2018). Advances in Meteorology, 2019.

Zhang, C., Song, Z., Zhuang, D., Wang, J., Xie, S., Liu, G. 2019a. Urea fertilization decreases soil bacterial diversity, but improves microbial biomass, respiration, and N-cycling potential in a semiarid grassland. Biology and Fertility of Soils, 55(3), 229-242.

Zhang, H., Zhao, Z., Li, S., Chen, S., Huang, T., Li, N., Yang, S., Wang, Y., Kou, L., Zhang, X. 2019b. Nitrogen removal by mix-cultured aerobic denitrifying bacteria isolated by ultrasound: Performance, co-occurrence pattern and wastewater treatment. Chemical Engineering Journal, 372, 26-36.

Zhou, J., Jiang, X., Wei, D., Zhao, B., Ma, M., Chen, S., Cao, F., Shen, D., Guan, D., Li, J. 2017. Consistent effects of nitrogen fertilization on soil bacterial communities in black soils for two crop seasons in China. Sci Rep, 7(1), 3267.

Appendix 3

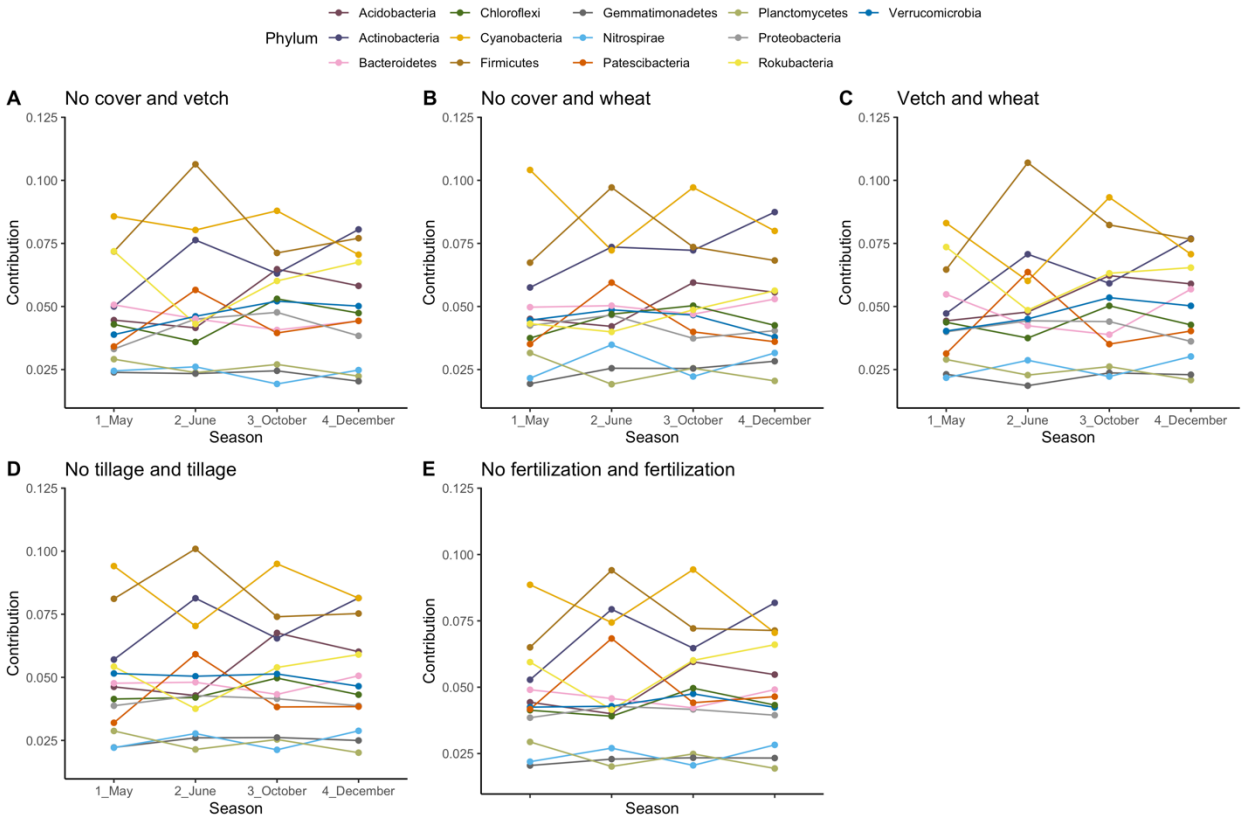


Figure 3.1 The contribution (%) of bacteria phyla to dissmilatory of pairwise comparsion among cover cropping, tillage, and fertilization.

Cover cropping (A, B, C) tillage (D), fertilization (E) and across four seasons based on SIMPER (Analysis of Similarity) analysis.

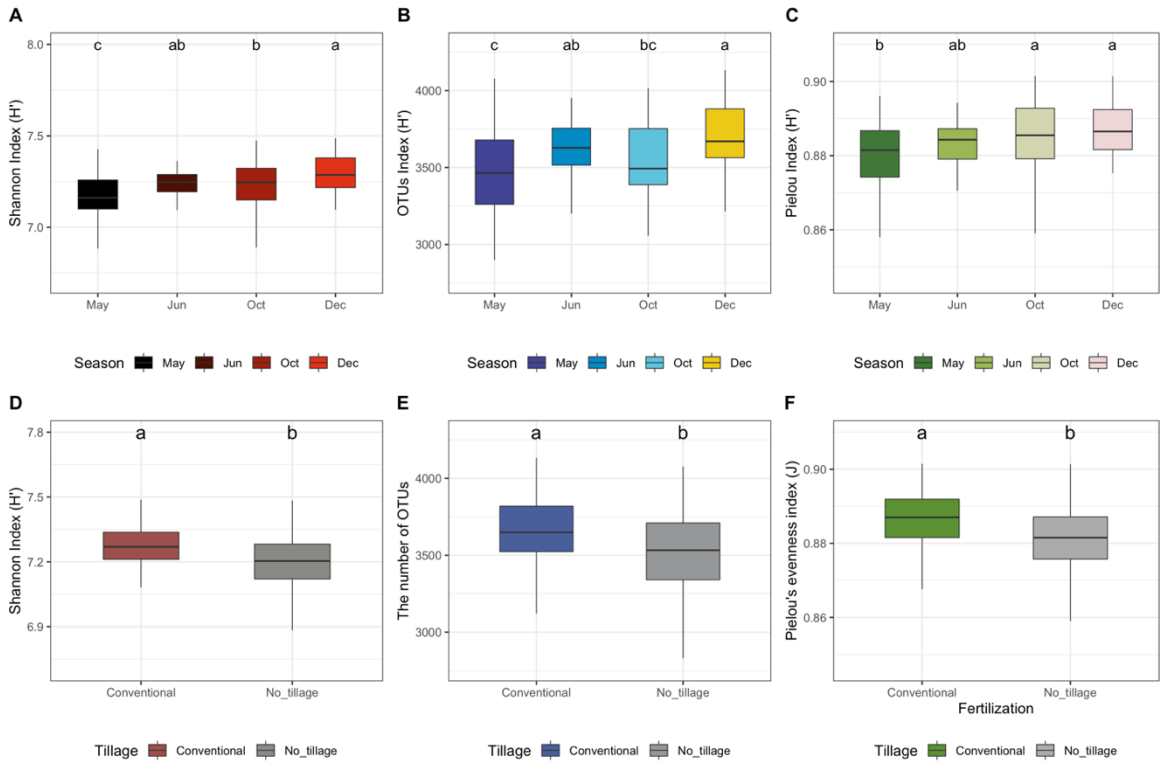


Figure 3.2 Boxplot of bacteria richness and evenness across four sampling seasons and tillage management.

Shannon (A and D), the number of OTUs (B and E), and Pielou's evenness (C to F) indices represented bacteria alpha-diversity, richness, and evenness. Minimum value, first quartile (Q1), median, third quartile (Q3), and maximum value were shown in the plot. The box shared different letters above mean two treatments were significant different ($p < 0.05$).

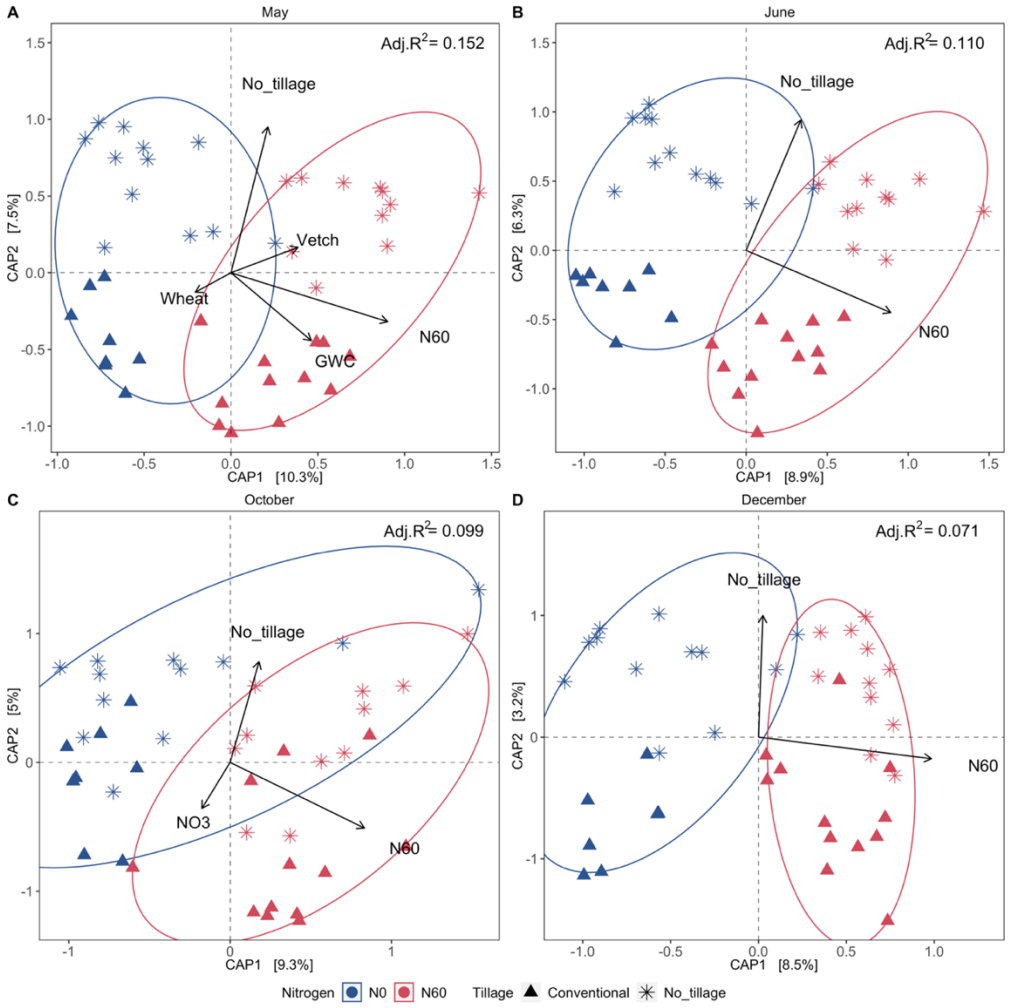


Figure 3.3 Canonical analysis of principal coordinates analysis (CAP) across four seasons under tillage, N fertilization, and cover cropping.

Fig. 3.3A to D represent the samples collected in May, June, October and December, respectively. Colors represent the fertilization, and shapes represent the tillage treatment. The significant environmental indicators were showed as arrows ($p < 0.05$). The Bray-curtis dissimilarity were calculated to estimate the variation among samples. GWC was the gravimetric water content. NO3 was nitrate-N in ppm. N60 represented inorganic N fertilization. Vetch and

wheat were two cover cropping types. Adjusted R square indicate total percentage explanation by significant indicators within each season, which were shown on the right corner of each plot.

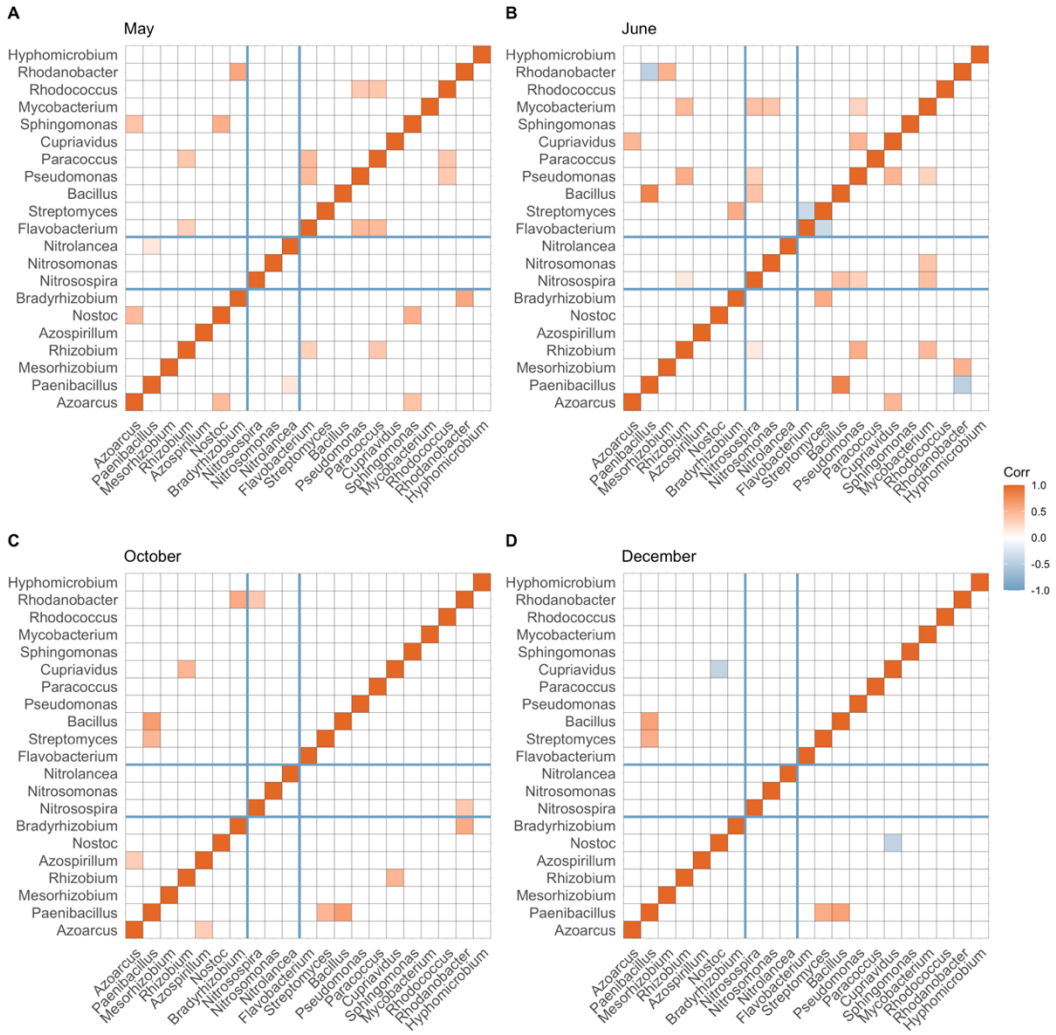


Figure 3.4 Spearson's correlation among bacteria at genus level involved in N-fixation, nitrification, and denitrification.

Relative abundance of N-fixation (*Azoarcus* to *Bradyrhzobium*), nitrification (*Nitrosopira* to *Nitrolancea*), and denitrification (*Flavobacterium* to *Hyphomicrobium*) across four sampling seasons, significant correlations ($p < 0.05$) were colored by red (positive correlation) and blue (negative correlation). The darker of color, the greater absolute value of correlation coefficient. False discovery rate was controlled using Benjamini and Hochberg method (1995). The number within cycle represent the correlation coefficient (p-adjusted < 0.05).

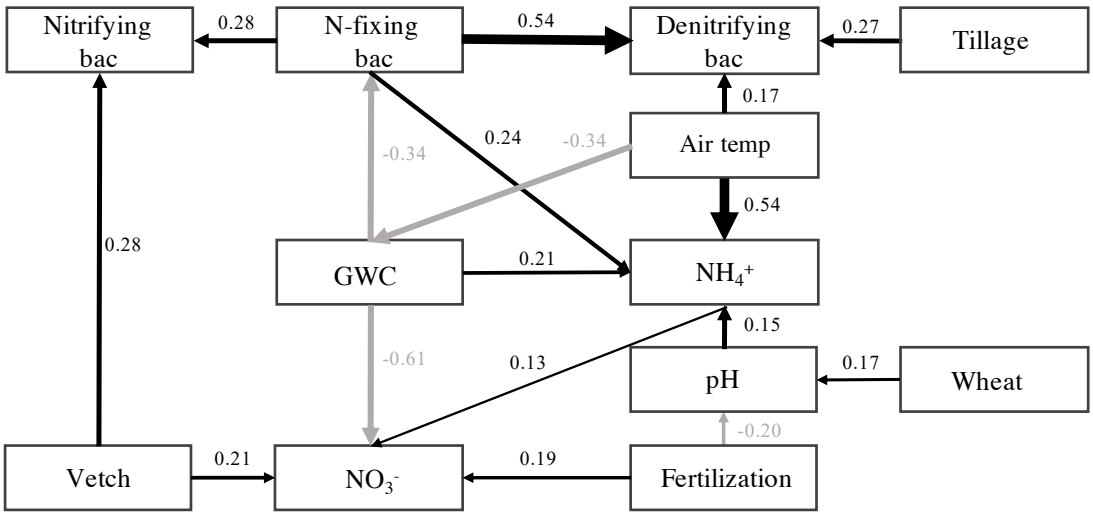


Figure 3.5 Structure equation model for N-fixing, nitrifying, and denitrifying bacteria.

Boxes represent variables. Single-headed arrows represent causal relationships, and double-headed arrows correlations. Black arrows indicate positive effects, and grey arrows negative effects. No arrows suggest no significant relationships between variables. All presented relationships are significant at $p < 0.05$. Numbers beside each arrow are standardized path coefficients (i.e., effect sizes). See Table 1 for standardized total effects. The "Vetch" and "Wheat", "Tillage", and "Fertilization" were presenting the cover cropping, conventional tillage,

and inorganic N fertilization treatment, respectively. Soil pH (pH), Gravimetric water content (GWC), ammonia-N (NH_4^+), and nitrate-N (NO_3^-), air temperature (month average ahead of sampling date) were as parameter presented in rectangular box.

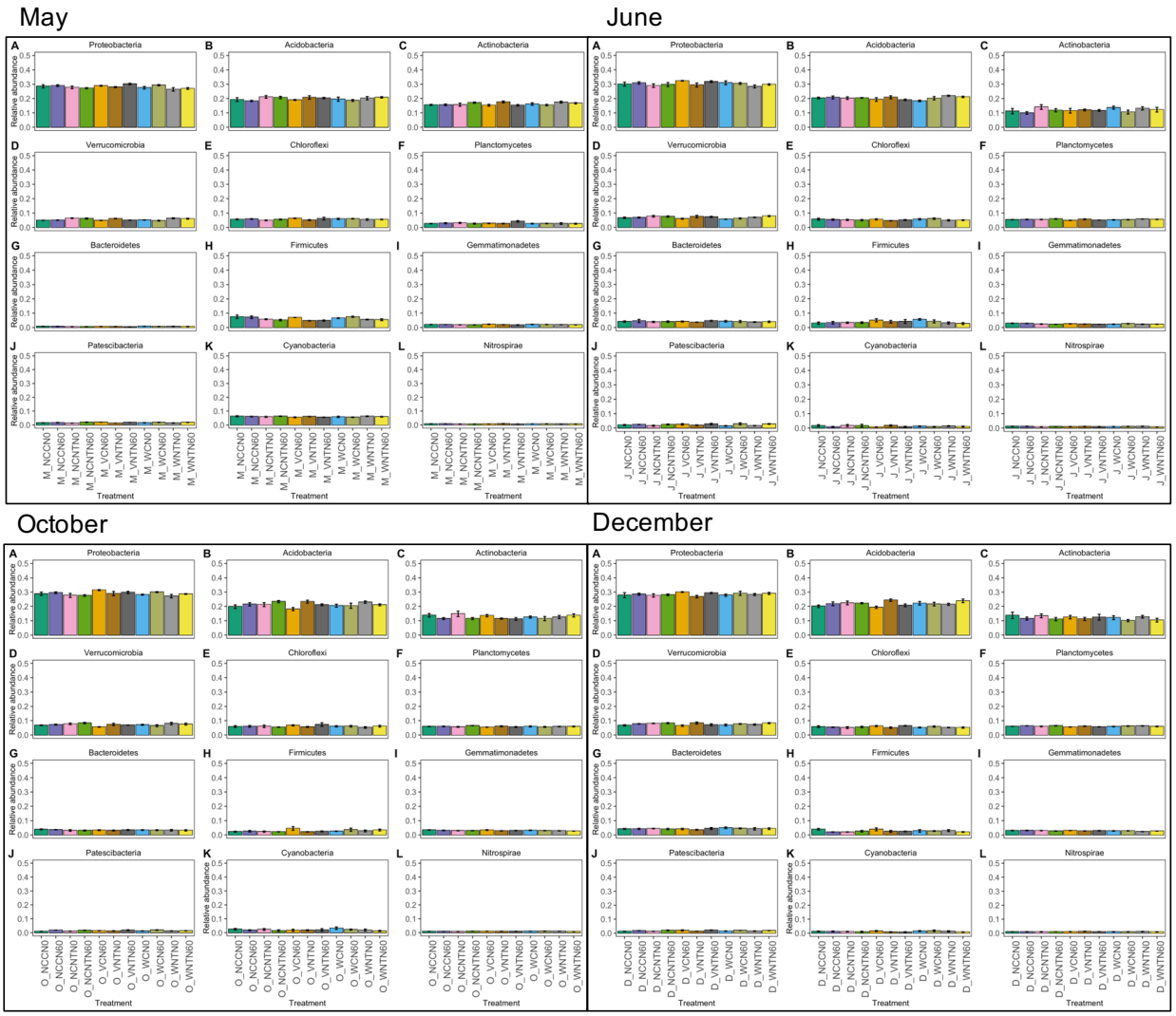


Figure 3.6 The relative abundance of dominate bacteria phyla collected in May, June, October, and December.

Table 3.1 Results of three-way ANOVA testing based on RCBD model of bacterial alpha diversity.

Shannon index				
Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Season	3	82.67	6.49	***0.0005***
Nitrogen	1	3.887	0.05	0.8353
Cover	2	13.76	0.61	0.5586
Tillage	1	24.51	9.88	***0.0043***
Nitrogen*Season	3	82.64	0.59	0.6264
Tillage*Season	3	82.64	0.95	0.4205
Cover*Season	6	87.7	0.57	0.7508
Nitrogen*Cover	2	14.32	1.6	0.236
Nitrogen*Tillage	1	23.32	0.2	0.6595
Cover*Tillage	2	24.03	0.53	0.595
Nitrogen*Cover*Season	6	87.7	0.91	0.4928
Nitrogen*Tillage*Season	3	82.67	0.46	0.7082
Cover*Tillage*Season	6	87.7	0.11	0.9945

OTUs				
Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Season	3	86.6	5.45	***0.0018***
Nitrogen	1	6.993	0.25	0.6296
Cover	2	13.54	0.37	0.6976
Tillage	1	38.02	9.93	***0.0032***
Nitrogen*Season	3	86.58	0.69	0.5614
Tillage*Season	3	86.58	0.31	0.8195
Cover*Season	6	91.81	0.3	0.9373
Nitrogen*Cover	2	14.14	0.86	0.4443
Nitrogen*Tillage	1	36.42	0.01	0.9434
Cover*Tillage	2	37.33	0.16	0.8569
Nitrogen*Cover*Season	6	91.81	0.59	0.741
Nitrogen*Tillage*Season	3	86.6	0.06	0.9795
Cover*Tillage*Season	6	91.81	0.13	0.9919

Pielou's evenness

Table 3.1 Continued

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Season	3	85.22	4.71	***0.0043***
Nitrogen	1	23.18	0.01	0.9343
Cover	2	17.87	1.06	0.3657
Tillage	1	30.23	6.66	***0.015***
Nitrogen*Season	3	85.19	0.99	0.4004
Tillage*Season	3	85.19	1.08	0.3626
Cover*Season	6	90.23	0.86	0.5267
Nitrogen*Cover	2	18.13	3.18	0.0655
Nitrogen*Tillage	1	28.26	0.71	0.4065
Cover*Tillage	2	29.48	1.87	0.1712
Nitrogen*Cover*Season	6	90.23	0.73	0.629
Nitrogen*Tillage*Season	3	85.22	0.72	0.5421
Cover*Tillage*Season	6	90.23	0.3	0.9362

Table 3.2 PERMANOVA analysis for each season

May							
Df	SumOfSqs	F	Pr(>F)			$r.squared	
Nitrogen	1	0.3728	3.7754	0.0001	***	[1]	0.2530885
Tillage	1	0.3281	3.3226	0.0001	***		
Cover	2	0.2602	1.3175	0.0297	*	$adj.r.squared	
GWC	1	0.1622	1.6425	0.0101	*	[1]	0.1521545
Residual	37	3.6534					

June							
Df	SumOfSqs	F	Pr(>F)			$r.squared	
Nitrogen	1	0.4323	4.0821	1.00E-04	***	[1]	0.1527435
Tillage	1	0.3429	3.2381	1.00E-04	***		
Residual	40	4.2359				$adj.r.squared	
						[1]	0.1103807

Oct							
Df	SumOfSqs	F	Pr(>F)			$r.squared	
Nitrogen	1	0.4632	4.1431	0.0001	***	[1]	0.1635279
Tillage	1	0.2725	2.4377	0.0006	***		
NO3	1	0.226	2.0212	0.0055	**	$adj.r.squared	
Residual	39	4.3599				[1]	0.0991839

Dec							
Df	SumOfSqs	F	Pr(>F)			$r.squared	
Nitrogen	1	0.4147	3.7431	0.0001	***	[1]	0.1164395
Tillage	1	0.1628	1.4694	0.0285	*		
Residual	39	4.3206				$adj.r.squared	
						[1]	0.0711287

Chapter 4 Identification of novel viruses and its microbial host from soils with long-term fertilization and cover cropping management

A version of this chapter is in preparation for submission to Environmental Microbiology

by Ning Duan [a], Mark Radosevich [a], Jie Zhuang [a, c], Jennifer M. DeBruyn [a], Margaret E. Staton [b], Sean M. Schaeffer [a,*]

Contribution statement

Ning Duan carried out the experiment, molecular experiments, and all data processing and statistical analysis. Jie Zhuang, Mark Radosevich, Jennifer M. DeBruyn , Margaret E. Staton, Sean M. Schaeffer are the responsible for the project and help with revision of manuscript.

[a] Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

[b] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, United States

[c] Center for Environmental Biotechnology, The University of Tennessee, Knoxville, TN 37996, United States

* Corresponding author. Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

Abstract

Microbes play a central role in processing carbon and nitrogen in the soil systems. Viral infection-induced microbial mortality could impact overall microbial diversity and ecosystem function. Little is known about the roles of soil viruses on biogeochemical cycling due to the challenge of identifying viruses from agricultural soils with more diversity of microbes. In this study, we recovered 260 viral operational taxonomic units (vOTUs) from twelve agricultural soil samples across four fertilization and cover cropping treatments. Soil was collected from a field with continuous cotton production and conservation agricultural management for more than 40 years. Only ~6% of vOTUs were clustered with the viruses in the RefSeq database. Of the 14% of total vOTUs that were linked to the microbial host populations covered the key carbon cycling and nitrogen cycling taxa including *Acidobacteriota*, *Proteobacteria*, *Verrucomicrobiota*, *Firmicutes*, and ammonia oxidizing archaea (AOA) *Nitrososphaeria* (*Thermoproteota*) were identified. Viral diversity, community structure, and apositive correlation between the abundance of virus and host indicate that viruses and microbes are more sensitive to nitrogen fertilization than cover cropping treatment. Soil phages can contain the genes that may adapt to their hosts and improve their fitness when the viruses are in a lysogenic phase. This is, potentially, an effective way for viruses to survive in the heterogeneous soil environment and may also influence C and N cycling through control of microbial function (e.g., *Chthoniobacterales and Nitrososphaerales*). These findings provide an important foundation for soil viral ecology, and likely impact the function of the soil system under long-term conservation agricultural management.

4.1 Introduction

Microbial diversity is an important biological indicators of soil health manifested by nutrient cycling and reflects the resilience and resistance of the soil to environmental perturbation (Van Bruggen & Semenov, 2000; Torsvik & Øvreås, 2002; Jackson et al., 2003; Allison & Martiny, 2008; Cardoso et al., 2013; Trivedi et al., 2016). Soil is known to harbor abundant and diverse viral assemblages, but their ecological role and influence on microbial processes has not been fully elucidated. In aquatic environments, viruses are a major driving force regulating microbial biomass and influencing the microbial food webs (Fuhrman, 1999a; Wommack et al., 2015). It has been proposed that much of dissolved organic carbon resulting from bacterial lysis due to phage infection is recycled in microbial biomass instead of transferring to higher trophic levels, this repeating cycling is called “microbial (bacterium–phage–DOC) loop” or “viral shunt”(Azam et al., 1983; Fuhrman, 1992; Kimura et al., 2008a). In soil, an analogous conceptual model was proposed for viral lysis impact and its impact on the distribution of soil labile carbon and recalcitrant carbon pools (Williamson et al., 2017a).

Appropriate utilization of inorganic N fertilization and cover cropping are common strategies for improving soil quality and productivity (Hobbs et al., 2008; Mbuthia et al., 2015b). Inorganic nitrogen fertilizers, along with cover cropping, increase inputs soil organic matter, change the soil moisture content, pH and other soil properties (Peoples et al., 2017; Romdhane et al., 2019; Smith et al., 2019). All these management activities have important effects on influencing bacteria diversity and community structure (Rousk et al., 2010b; Dai et al., 2018; Zhang et al., 2019a). Soil pH influenced by fertilization, organic matter charges, and host communites can

affect viral adsorption and desorption which potentially shift viral communities structure (Chen et al., 2014). However, we have less knowledge on response of viral communities under a long-term tillage and N fertilization management.

Research addressing the role of viruses in soil ecosystems has steadily gained momentum (Emerson et al., 2018; Trubl et al., 2018a; ter Horst et al., 2020; Trubl et al., 2020). Evidence is steadily accumulating that viruses can impact soil microbes through predation control (called "top-down") in the soil environment (Allen et al., 2010a; Guemes et al., 2016; Williamson et al., 2017a). In soil systems, viral abundances in soil can exceed 10^9 VLP g-1 in agricultural soil, which is often an order of magnitude greater than microbial abundance in soil (Wommack et al., 2000; Lauber et al., 2013; Adriaenssens et al., 2015; Cobián Güemes et al., 2016). However, soil and aquatic viral ecology appears to be a much greater variation in the ratio of virus abundance to microbial abundance (VMR) in soil (Williamson et al, 2017). The cause(s) for the greater variation in soil VMR remain unknown but may in part be due to a longer natural persistence of soil viruses due to adsorption to soil particles. Viral lysis contributed to 16%-48% of prokaryotic cell mortality in sediments (Hewson & Fuhrman, 2003; Danovaro et al., 2008). The extent of viral mediated host-cell lysis in unknown in soil environments.

The reproductive cycle of viruses falls into two general categories lytic or lysogenic cycle. A virulent (lytic) phage exploits the biosynthetic machinery of the host to produce, assemble and release daughter phages by lysing the host cells (Wang et al., 2018b). The phage nucleic acid integrated into the host genome, and as prophage replicates as the host grows and divides. The initiation of the lytic cycle from the lysogenic cycle can be triggered by environmental factors,

such as ultraviolet (UV), toxins, warming, and host cell density dependence (Williamson et al., 2007; Fornelos et al., 2018). Lysogeny can be a common lifestyle for viruses in soil (Marsh & Wellington, 1994). The genetic communication with capacities of recombination encoded by prophages is the source of viral diversity (Menouni et al., 2015). Among the most common host bacteria for soil viruses are *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Nitrospirae*, *Desulfobacterota* (Williamson et al., 2017a; Emerson et al., 2018; Trubl et al., 2018a; ter Horst et al., 2020).

Understanding virus-host linkage between host and virus is important to regulate the capability of viruses to influence bacterial community and functions (Weinbauer & Rassoulzadegan, 2004b; Mann, 2005). Viral genomes contain the host-derived functional gene that might reprogram the host metabolic process to favor the phage fitness and reproduction (Breitbart et al., 2007). It is thought that auxiliary metabolic genes (AMGs) in viruses tend to sustain or supplement host essential metabolism to help successfully infect the host cells and produce more viruses per cell (Enav et al., 2014; Paul, 2008). These auxiliary metabolic genes (AMGs) are known to participated in the carbon and energy metabolisms (Enav et al., 2014; De Smet et al., 2016; Gazitúa et al., 2020). For example, central carbon metabolic pathways, electron transport, and photosystem genes in viruses may improve the nucleotide and energy production during the infection (Sharon et al., 2011; Trubl et al., 2018a). Glycoside hydrolase genes may contribute to organic carbon degradation of monosaccharides and oligosaccharides into simple sugars (Emerson et al., 2018; ter Horst et al., 2020). Many nitrogen cycling-related functional genes, which are involved in denitrification, nitrification, assimilatory nitrate reduction, and nitrite transport were discovered in marine systems (Gazitúa et al., 2020). Polysaccharides were

correlated to the variability of the host cell membrane and important receptor structure for bacteriophage recognition (Datta et al., 1977; Bertozzi Silva et al., 2016; Knecht et al., 2020).

Several relatively low-resolution techniques for studying the viral diversity in soil are low-resolution technique, such as Random Amplification of Polymorphic DNA-Polymerase chain reaction (RAPD-PCR) RAPD-PCR have been applied. RAPD-PCR is a low-cost, genetic fingerprinting tool has been applied to examine the genetic diversity of viral diversity (Srinivasiah et al., 2013; Liang et al., 2019), though no taxonomic information can be obtained. Isolation of viruses from soil and whole-genome sequencing is lab-intensive and limited to cultured microbes. The most widely used approach to study virosphere in soils are whole soil metagenome sequencing of virus-enriched metagenomes (i.e., viromes) from environmental samples (Emerson et al., 2018; ter Horst et al., 2020). Viral information from bulk-soil metagenome sequencing can be obtained from a variety of bioinformatic tools designed to search and identify viral from non-viral genetic information (Emerson et al., 2018; Trubl et al., 2018a; Trubl et al., 2020).

Here, we exploit the bulk-soil metagenomes prepared from twelve samples across four agricultural treatments to (1) recover vOTUs from soils and investigate the effect of long-term cover crop (e.g., vetch) and fertilization management on viral community composition, and (2) identify bacteria and archaea host for the viruses, (3) identify how N fertilization and cover cropping impact the link between viruses and their host, The results are expected to shed light on the soil virosphere and improve the understanding of the ecological roles of soil viruses in agroecosystems.

4.2 Material and methods

4.2.1 Site description and sample collection

The on-going long-term conservation management experimental site was established in 1981 and has been continuously operated to present. The continuous cotton production experiment in a randomized complete block (RCBD) with a split-split plot design. Inorganic nitrogen fertilizer (ammonium nitrate, NH_4NO_3) was applied at two nitrogen levels (0 and 67kg/ha) as the main plots and were divided into sub-plots that contained two levels of cover crop treatments (no cover and hairy vetch, *Vicia villosa* Roth) and no-tillage treatment. All four treatments with three replications from three distinct plots were sampled on May, 21st, 2019, shortly after burn-down of the cover crop and just before planting in spring. The samples were coded with the following form: “type of cover crop”, “no-tillage”, “fertilization or not” and “serial number of replicates”, e.g. NCNTN0_1 represents no-cover, no-tilled, and no fertilization treatment replicate #1; VNTN60_4 is vetch-covered, no-tilled with fertilization of 67 N kg/ha, replicates #4. Bulk soil metagenomic sequencing was applied on 12 samples from selected treatments.

4.2.2 DNA extraction, library construction, and metagenomic sequencing

Soil samples were collected from a depth of 0 to 10 cm at a distance of 10-15 cm from the center of crop row using a 2.5cm diameter soil probe. About 10-15 subsamples were randomly taken within each plot. The subsamples from each plot were thoroughly mixed into one composite sample with rock and roots removed and then placed into a zip-loc bag. The samples were

transported in a cooler with ice bags within 24 hours to the lab freezer (-20 ℃). Prior to experiment, fine rocks, roots, and other debris were removed by passing each composite soil sample through a 2mm sieve to remove fine rocks, roots, and other debris in the lab.

Total soil DNA was extracted using DNeasy® PowerLyzer® Powersoil® kit (REF 12855, QIAGEN) from 0.25 g soil per sample following the manufacturer's instructions. The extracted DNA was dissolved in sterile DNA-free PCR grade water. Qubit™ 1 × dsDNA HS (High-Sensitivity) Assay Kit (Invitrogen, ref: Q33230) was used to test the quality of whole soil DNA on Qubit 2.0 Fluorometer (total 200 µl reaction). A260/A280 was measured using NanoDrop™ OneC Microvolume UV-Vis Spectrophotometer (Thermo Scientific™).

Genomic DNA libraries were constructed for sequencing on the Illumina platform using KAPA Library Preparation Kit (Kapa Biosystems, Woburn, MA). First, DNA was fragmented with the Covaris E210. Then libraries were prepared using a modified version of the manufacturer's protocol. DNA was purified between enzymatic reactions and size selection of the library was performed with SPRI select beads (Beckman Coulter Genomics, Danvers, MA). For indexed samples, the PCR amplification step was performed with primers containing dual unique barcode sequences 8 nt in length.

Libraries were assessed for concentration and fragment size using the DNA High Sensitivity Assay on the LabChip GX (Perkin Elmer, Waltham, MA). The library concentrations were also assessed by qPCR using the KAPA Library Quantification Kit (Complete, Universal) (KAPA Biosystems, Woburn, MA). The libraries were pooled and sequenced on Illumina NovaSeq6000

S2 using 150bp PE reads (0.5 S2-lane, 900M read pairs, 275 Gbp yield average, Illumina, San Diego, CA) at the institute for genome sciences, school of Medicine at the University of Maryland.

4.2.3 Reads pre-processing and assembly

Cutadapt (version 1.18) was used to remove the adapters (smallRNA, Illumine, Nextera), the Quality Phred score cutoff was 20, Maximum trimming error rate was 0.1, the reads shorter than 50bp were discarded (you could base on metawrap pipeline)(Martin, 2011). *De novo* assembly with MEGAHIT (Li et al., 2015; Li et al., 2016). QUAST(v.5.0.2) was used to estimate contigs statistics (Mikheenko et al., 2018).

4.2.4 Viral population recovery reads mapping, and taxonomy identification

The assemblies in each sample were clustered with PSI-CD-HIT implementation of BLASTn to cluster contigs with a global identity threshold of 0.95 to remove the redundant sequences (dereplication) (Li et al., 2002; Li & Godzik, 2006; Evans & Denef, 2020). Non-redundant assemblies were processed by VirSorter (v.1) and Deepvirfinder (Roux et al., 2015a; Ren et al., 2018). The category 1, 2, 4, 5 viral contigs were identified by Virsorter were retained. The contigs with DeepVirfinder scores higher than 0.9 and $p < 0.05$ and VIBRANT (v1.2.0) were considered (Kieft et al., 2020). All the contigs were manually combined in each sample. All the contigs lengths greater than 10,000 bp were clustered into vOTUs using CD-HIT with global identity threshold of 0.95, alignment coverage for shorter contigs length more than 85%. The bbmap was used to map short-reads sequences in each sample to the updated viral database

(PIGEON v1.0 plus the viral contigs (step 2) we assembled across the samples)(Bushnell, 2015; ter Horst et al., 2020). Sam file with alignment information of each sample was transformed to the bam format and bam files were sorted and indexed by SAMtools (Li et al., 2009) and BEDtools (Quinlan & Hall, 2010) to parse the bam file and for downstream generation of coverage table by BamM. The vOTU table was normalized based on the contig length and library size to make it comparable among samples (Emerson et al., 2018; Trubl et al., 2018a; ter Horst et al., 2020). A square-root transformation was applied on abundances of vOTUs for statistical analysis.

Viral taxonomy assignment was by building the gene-sharing network in vContact2 (Zablocki et al., 2019). The fasta file with nucleic acid sequences including the viral contigs in each sample assembled by MEGAHIT (deduplicated and updated PIGEON v1.0 database using the viral contigs we assembled across 12 samples in our study), this file was annotated by MetaProdigal (Hyatt et al., 2012), the one output file with amino acid annotation was fed into the vContact2 for taxonomy assignment. Nodes and edges represent viral genomes (or contigs) and significant similarity ($p < 0.05$) between the protein cluster (PC) profiles. The similarity between sequences depends on the number of shared protein clusters (Bolduc et al., 2017). The analysis was performed on 260 vOTUs generated by reads mapping across 12 samples in our study with vConTACT2. vOTUs were clustered with 2,617 viral genomes in the RefSeq database based upon shared protein clusters (O'Leary et al., 2016). The shared genus level viral clusters were selected manually for further analysis.

4.2.5 Metagenome-assembled genome (MAG) construction

Metabat2 (v2.12.1) (Kang et al., 2019), Maxbin2 (v2.2.6)(Wu et al., 2014; Wu et al., 2016), and concoct (1.0.0) (Alneberg et al., 2013) within MetaWRAP (v1.3.2) (Uritskiy et al., 2018) were applied on the microbial contigs, which were assembled by MEGAHIT. The contigs greater than 2.5kbp on each sample were recovered the microbial draft genomes. CheckM (Parks et al., 2015) was used to checking quality of the genome, and genomes were retained with quality score ≥ 50 (Parks et al., 2017). MAGs were deduplicated by dRep (v3.0.0)(Olm et al., 2017), and MAGs taxonomic affiliations were classified with the GTDB-tk (v1.3.0) workflow (Chaumeil et al., 2020).

To estimate the relative abundance of each microbial population, the short-read sequences were mapped to assembled contigs before binning as described above. First, the contigs in all the samples were combined, and all the contigs were clustered using CD-HIT with global identity threshold of 0.95 and alignment coverage for shorter contigs length more than 85%. The renamed, deduplicated, and sorted fasta file as references contigs were indexed by bbmap, which was similar to the approach for the viral abundance calculation. Third, all the clean reads in every sample were mapped back to the reference contigs by bbmap. This step was to generate the coverage table of each sample. The final OTU table was generated by average coverage weighted by total contig length in base pairs and calculating the average of all its binned contigs coverages. The MAGs abundance table was normalized by the read depth for each sample following the same procedure with the vOTU table described above. The low coverage (< 0.25

×) of vOTU contigs was filtered and converted to zero (Emerson et al., 2018; ter Horst et al., 2020). A square-root transformation was applied on abundance of MAGs for statistical analysis.

4.2.6 Building phylogenetic tree for the host genome

Alignment of forty three conserved maker-genes with largely congruent phylogenetic histories, and concatenated protein sequences of single-copy genes generated by CheckM (v1.1.3) were used to build an unrooted phylogenetic tree (Parks et al., 2015). The maximum likelihood phylogenetic tree were built by using Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) as the distance method, 500 bootstraps as phylogeny test were constructed with the Le_Gascuel_2008 substitution model (Le & Gascuel, 2008) by MEGA X (Kumar et al., 2018; Stecher et al., 2020). The percentage of the trees clustered, and the genetic divergence was shown within the tree.

4.2.7 Virus–host linkage based on CRISPR arrays and BLAST

In this study, we used two methods to identify the link between the virus and their host. First, CRISPR repeats, and spacer arrays were applied on the 12 samples by Crass v1.0.1 with default settings individually (Skennerton et al., 2013), viral contigs and spacers sequences (protospacer-spacer matches) recovered from metagenomes were compared by BLASTn (blastn-short task, percentage identity 0.95, and E-value threshold of 10e-5, mismatch ≤ 1) (McGinnis & Madden, 2004). The direct repeats were chosen based upon the spacers matches in viral genomes, and BLASTn were used against direct repeats to bacterial and archaeal genomes (blastn-short task,

percentage identity 1, and E-value threshold of 10e-10, mismatch ≤ 1) to link the virus with a host.

The second approach was blastn, identified vOTU nucleotide sequences blast against to the MAGs we found in this study. The vOTUs were retained if the bit score > 50, E-value < 10e-3, ≥ 70% of average nucleotide idenity (ANI) were estimated using FastANI (Jain et al., 2018). The hits that were ≥ 2,500 bp and covered ≤ 90% of viral contigs in microbial genomes were considered for the most confident provirus predictions, the hits that covered > 90% were considered less confident (Roux et al., 2016; Emerson et al., 2018; Trubl et al., 2018a). Pearson's product moment correlation was conducted on both viral and their potential hosts normalized abundances; Normality Tests were conducted using the Shapiro-Wilk test prior to correlation analysis (Shapiro & Francia, 1972). The vOTU were categorized as temperate or virulent based upon whehter an integrase is identified (Kieft et al., 2020).

4.2.8 Statistical analysis

Shapiro - Wilk Normality Test was performed to check the normality of normalized mean coverage depth of vOTUs and genomes, diversity indices (Shapiro et al., 1968). A square-root transformation was performed to correct the normality of the data. A mixed effect model was built, the block was regard as random effect and fertilization and cover crops were treated as fixed effect. Type II Wald Chi-square tests were applied on the mixed model to test an alternative hypothesis. Tukey's HSD (Honest Significant Difference) was performed for the post-hoc test (Abdi & Williams, 2010).

4.3 Results

4.3.1 Dataset overview and agricultural soil viral population (vOTU) recovery

Using bulk-soil metagenome sequencing to distill the soil virus information is one of the important approaches to investigating soil virus community and their ecological roles (Trubl et al., 2018a). The sequences of the dsDNA viruses were extracted by recently developed bioinformatics tools as described above from 12 agricultural soil samples including four treatments. To quantify dsDNA viral diversity in long-term managed agriculture soil, we collected 12 soil metagenomes from the cores gathered from the long-term tillage, fertilization, and cover cropping plots in West Tennessee. Approximate sequencing depths of 20 Gbp paired reads per metagenome were obtained by Illumina NovaSeq 6000 platform. Over 99% of raw reads per sample were passed through the quality control steps. After quality filtering, metagenome per sample yielded 69,652,934 paired reads per library on average for 12 samples collected from the 4 treatments (Figures S4.1 and S4.2).

The total of 260 viral OTUs (vOTUs) were predicted by VirSorter, DeepVirfinder, and VIBRANT pipline. A total number of high-quality pair-end reads that were obtained ranged from 51,805,430 to 90,119,576 with an average of 1.15% to 1.91% were assembled into contigs greater than 1kbp. The reads were assembled to an average of 4,922 on average soil viral contigs and ranged from 27,152 to 86,666 bp (Table 4.1). All soil viral contigs from the 12 samples clustered into 56,780 unique viral contigs, but only 241 viral contigs was greater than 10 kb. No plasmids were identified in 241 vOTUs based upon manual screening. Finally, the viral OTU

table with 260 of vOTUs were generated by mapping reads to the updated PIGENON database (including our 241 viruses found in our soil samples). All the 260 vOTUs recovered in this study included the 10 named "alaska_puertorico" contigs obtained from permafrost (Trubl et al., 2019), only 1 viral contigs names "EarthsVirome_48737" and 14 of viral contigs named "gary_all20" (IMG/VR) and analyzed by Paez-Espino et al., 2016 (Paez-Espino et al., 2016), 2 viral contigs "SPRUCE_viral_seq" orginated from Peatlands in northern Minnesota (ter Horst et al., 2020), and 1 viral contig from named with "virsorter_curated" recovered from the publicly available microbial genomes (RefSeq and WGS databases) (Roux et al., 2015b), and rest of 232 out of 260 vOTUs (89.23%) were without significant hits to the databases we examined (Table S4.2). The result indicated that soil virosphere is undersampled and different soil types may harbor different viruses.

4.3.2 Gene-sharing network for viral taxonomy assignment

A gene-sharing network was built to assign viral taxonomy due to the lack of universal marker genes in viruses (Fig. 4.1). The 260 of vOTUs were clustered with 2,617 prokaryotic viral genomes in the RefSeq database (v94). The 107 out of 260 vOTUs passed the vConTACT2 threshold and presented in the networks. 30 vOTUs as outliers in the networks were removed, and 77 of vOTUs were clustered, while 1,658 nodes and 32,667 edges were left in the networks. Only 6% of vOTUs identified in this study were similar to the viruses in the database, and 11 viral clusters (VCs) were related to reference databases (Fig. 4.2B).

The vOTU_30 was grouped the same cluster (VC_225_1) as 5 Cronobacter_phage (VC_225_0) classified as g_*Cr3virus* (f_ *Myoviridae*), indicating that the two genomes were highly related at the subfamily level, which was *Vequintavirinae*. The vOTU_63 and vOTU_93 (VC_69) clustered with 28 viruses belonging to the g_*Peduovirus* affiliated with the *Myoviridae* family, and they are all of which were P2-like viruses.

With high probability, vOTU_229 was assigned to the *Siphoviridae*, which were grouped with Pseudomonas_phage_phiPSA1, Sinorhizobium_phage_phiLM21, and Vibrio_phage_SHOU24. vOTU_69 clustered with Ralstonia_phage_RSK1 in the same subcluster (VC_164_0), which belongs to the f_*Podoviridae*, but the genus was unclassified due to the limitation on identification of genes. vOTU_100 and vOTU_67 connected with 2 viruses and were assigned to *Bpp1virus*, others were unclassified, but they grouped within the lineage of *Podoviridae.*

4.3.3 Distribution and diversity of vOTUs across treatments

There were 8 vOTUs shared between no cover crops and vetch with no fertilization, and 19 vOTUs were shared by no-cover crops and vetch with fertilization. Only 4 vOTUs were shared by fertilization or not with vetch covered, and 3 were shared by the fertilization or not without vetch covered (Fig. 4.2A). These results suggested that fertilization might create a greater influence on shaping viral community than cover cropping. More unique vOTUs were found in no cover with no fertilization and vetch with fertilization treatment compared to no cover with fertilization and vetch without fertilization practices.

After annotating 260 vOTUs, only 8.46 % (22 vOTUs) were carried with recombinase or recombinase-like protein, and 13.07% (34 vOTUs) were identified as prophage by BLAST and CRISPR arrays (Table S4.3). Here, temperate phages take 31.57 %, 28.57 %, 33.27%, and 24.44% in no cover without and with fertilization, vetch without fertilization and with fertilization, respectively. The percentage was greater in soil without fertilization compared with fertilization (Table S4.7).

The diversity analysis revealed that soil with fertilization contains a significant greater number of vOTUs than no fertilization soil ($p < 0.05$, Table S4.8). Also, the diversity indices have shown that Shannon ($p < 0.01$) was significantly greater in fertilized soil than without fertilization, however, cover crops have no effects (Fig. 4.2C, Table S4.8). PCoA and two-way PERMANOVA were applied on vOTU table (number of permutations=2000, $p < 0.01$, Table S4.9), the 46.2% variation of the viral community could be explained by two axes of PCoA in total, suggesting that fertilization induced influence is more significant on composition and structure of viral community than cover cropping, there is no interaction effect between fertilization and cover crop treatments (Fig. 4.2D). No significant effects of POXC, inorganic N content, pH, soil moisture content on viral community structure were observed (Table S4.9).

4.3.4 The linkage between viruses and bacterial host

We employed CRISPR and BLAST to identify the link between the virus and its host. There are 52 spacers within 39 spacer groups identified from all 12 samples matched to our 260 vOTUs, and 39 direct repeats were blasted with the 13 bacterial and 7 of archaeal genomes. In our study,

more linkages between the virus and its host were detected by BLASTn with criteria described in the methods than by CRISPR-cas pattern. The bacteria hosts covered 4 bacterial phyla, and 5 genera (p_*Acidobacteriota*, g_ *PSRF01*; p_*Acidobacteriota*, g_*Gp1-AA122*; p_*Proteobacteria*, g_ *Kosakonia*; p_*Verrucomicrobiota*, g_*AV55*; p_*Firmicutes*, g_*Paenibacillus*_J) (Fig.4.3, Table S4.5). The archaea 5 hosts are all in the lineage of *Nitrososphaeraceae* (f_*Thermoproteota*) (Fig. 4.5).

Virus with the same genus infected the same microbial host. *Verrucomicrobiota AV55* is the proposed host of vOTU_153, VOTU_203, vOTU_81, vOTU_115, vOTU_163, vOTU_252. The vOTU_153, vOTU_81, and vOTU_252 were under same viral linkage at genus level. Novel vOTUs identified in our study which cannot aligned to PIGEON database are not related at the genus level, but probably related at the higher level (subfamily, family, order). They could infect the same genus or even same species of the host. For example, vOTU_14, vOTU_181, vOTU_3, vOTU_152 were proviruses in *Acidobacteriota PSRF01* in different species (bin.112.7, bin 37.3, and bin 37.7). vOTU_14 cannot be assigned taxonomy through gene-sharing networks. vOTU_3 did not cluster with vOTU_181, vOTU_14, and vOTU_152 at genus level, but they were all related to *Acidobacteriota PSRF01*. Also, the vOTU_161, vOTU_106, and vOTU_148 was connected to the *Firmicutes Paenibacillus*_J. vOTU_106 and vOTU_148 were novel viruses not related at genus level with vOTU_161. vOTU_19 and vOTU_48 cannot be assigned taxonomy based on current database but were related to *Acidobacteriota* Gp1-AA122 (Fig. 4.3, Table S4.5).

Viruses sharing the same the genus do not necessarily link to the host at the genus level. In this study, vOTU_63, vOTU_93 connected to *Proteobacteria Kosakonia cowanii* species. Although vOTU_63 and 93 were clustered together in VC_69 and belong to *Peduovirus* (or *P2virus*) in *Myoviridae* lineage, their host might be the *Kosakonia cowanii* or other species within *Proteobacteria* phyla (Table S4.10).

Two linear mixed-effects model was built to test the effect of fertilization and cover crops on the abundances of viruses and abundance of their linked host, respectively. The relative abundances of *Paenibacillus* (*Acidobacteriota*) ($p < 0.05$) and g_*AV55* (*Verrucomicrobiota*) ($p < 0.001$) increased in fertilization compared with no fertilization. For viruses, the relative abundances of vOTU_19, vOTU_203, vOTU_81, vOTU_115, vOTU_163, vOTU_252 were significantly increased in fertilization. No significant effect of cover crops on the abundance of hosts and viruses can be found (Fig. 4.3). Furthermore, based on the presumptions of Pearson's correlation, vOTU_203, vOTU_81, vOTU_115, vOTU_163, vOTU_252 were analyzed separately based on the different fertilization treatment. Similarly, the abundances between virus and host were strongly positive connected, Approximately, more than 90% of the variation of the *Verrucomicrobiota AV55* (or viruses) on abundance can be explained by variance of viruses (or *Verrucomicrobiota AV55*) on abundance and vice versa (Fig. 4.4).

4.3.5 The linkage between viruses and archaeal host

All of vOTUs connected to *Nitrososphaeraceae* cannot be assigned by the gene-sharing network due to its novelty or lack of virus hallmark genes. We observed that archaea within the

Nitrososphaeraceae genus were significantly increased under cover crops (e.g., g_*Nitrosocosmicus* bin 16.5) ($p < 0.05$), and under no fertilization (g_*UBA10452*, g_*TA21* and g_bin35.3) ($p < 0.05$). The correlation between vOTUs and archaea on abundance were positively correlated like bacterial phages in our study (Fig. 4.6). Moreover, it presented the strong correlation ($r > 0.70$, $p < 0.05$) across different treatments, indicating that the relationship between viruses and *Nitrososphaeraceae* was influenced by cover cropping management, as well as fertilization (Fig. 4.6).

4.4 Discussion

4.4.1 The limitations of using metagenomes to discover soil viromes

The recovery rate of vOTU from bulk soil metagenome might increase with increasing number of samples and/ or sequencing depth. About 2,699 vOTUs across 82 bulk soil metagenomes were identified from peatland in northern Minnesota, all these total soil metagenomes were sequenced from 6 to 15 Gbp on average per library on the NovaSeq platform (ter Horst et al., 2020). Stordalen Mire 1831 vOTUs were identified from 178 bulk soil metagenomes in northern Sweden permafrost soil with an average output of 8 Gbp per sample (Emerson et al., 2018; Trubl et al., 2018b). About 97 of vOTUs were identified from across 16 samples approximately 2 Gbp per library from bulk soil metagenome sequencing in agricultural soil with biochar-amended (Santos-Medellin et al., 2020). Based on the studies described above, vOTU yield rate ranged from (normalized per gigabase-pair of metagenomes) 1.30 to 3.29.

In this study, the high-quality sequences obtained from platform that reached up to approximately 10 Gbp paired reads per library, 260 vOTUs (including 232 novel vOTUs and 28 overlapped with PIGEON database) can be identified from 12 agricultural soil DNA samples, vOTU yield rate of this study was 2.17, which is a reasonable recovery rate for soil studies. However, viromes is a better approach for recovering more vOTU from soil compared with bulk soil metagenomes, it can have about three to thirty times more viral populations on per-sample (Trubl et al., 2018b; Santos-Medellin et al., 2020; ter Horst et al., 2020). The connection between viruses and its host can be obtained by analyzing bulk soil metagenome, viromes cannot be used to explore the relationship between virus and its host due to lack the host information. Lab intensive and low DNA yields would be the downside for viromic approach (Trubl et al., 2020). We would suggest the combining bulk soil metagenome and viromes to identify the link between virus and its host. A trade-off between number of samples and sequencing depth should base on the aim and budget of researcher's study.

In this study, the taxonomy of more than 90% of viruses in this study could not be assigned according to known bacterial viruses in the RefSeq database. This finding suggested that the soil environment is home to many diverse and yet to be classified viral taxa. The limited information in current viral databases prevent our understanding of soil viral communities (Williamson et al., 2017a; Trubl et al., 2018a; Trubl et al., 2020).

4.4.2 Taxonomic classification of viral OTUs

Most of the structural protein of vOTU_63 and vOTU_93 (e.g., baseplate protein, tail and head completion protein, tail tube protein, and phage major capsid protein) were similar to those of bacteriophage P2 (Linderoth et al., 1991). Very late expression factor 1(VLF-1), a member of the tyrosine recombinase family of proteins, serves the basic function during the late stage of DNA packaging and capsid assembly (Vanarsdall et al., 2006). This recombinase was detected in vOTU_63 and vOTU_93, indicating that the vOTU_63 and vOTU_93 are temperate phages.

A portal protein was annotated in vOTU_100, which functions as a channel for passage of viral DNA bidirectionally, in tailed bacteriophages. DNA can move in and out of the virus head using portal protein which also provides an attachment point for the tail apparatus (Lokareddy et al., 2017). vOTU_149 and Brevibacillus phage were in the same subcluster (VC_108_0), which was assigned to the genus *Jenstvirus* in the family *Siphoviridae*. vOTU_9 (Virsorter_curated_4650) clustered with Bacillus phage (Milani et al., 2018), were in the same subcluster (VC_118_0), which belongs to genus *Cecivirus* of the family *Siphoviridae*. vOTU_145 was in the VC_337_0 with Thermoanaerobacterium_phage, which is in the family *Siphoviridae*. Recombinase, *XerC*, was annotated in suggesting it may be a temperate phage (Bobay et al., 2013).

4.4.3 Inorganic N fertilization influence the viral community diversity and structure

Inorganic N fertilization is favorable to influence viral community diversity and have importantly effects on shaping viral community structure. It is more likely N fertilization

indirectly favors influence viral population by increasing the abundance of host communities, which indicated in Chapter 2(Chen et al., 2014). Also, the strongly positively correlation between abundances of *Verrucomicrobiota* and its vOTUs was observed, and fertilization influence the abundance of genre *PSRF01* affiliated with *Blastocatellia*, the abundance of *Blastocatellia* was correlated with nitrogen availability in soils (Ivanova et al., 2020), which might indirectly influence the viruses related to the *Blastocatellia*. All these results in this study also support the variation of viral population may directly result from the changes of host population or abundances under N fertilization.

Meanwhile, N fertilization may contribute to the temperate phages percentage increasing in without fertilization than with fertilization, suggesting that the inorganic N might result in proportion of phages exist as prophages in their respective microbial host. In the aspect of bacteria-to-phage communication, the surviving strategy of bacteria would change (e.g. endospores formation in *Firmicutes*) due to the lack of nutrients of their surroundings (Nicholson et al., 2000). Phages could respond to the bacterial signals and modifying the outcome of their infection from obligately productive to temporarily reductive (Abedon, 2009; Gabiatti et al., 2018). For example, phages could be packed into bacterial endospores and then use it as a viral genome protection shell to resist environment stress (Gabiatti et al., 2018). Spore formation for septum and coat assembly-related AMGs (*spoVS* and *whiB*) had been reported in viral genomes (Trubl et al., 2018a). This mechanism is supported by previous studies that lysogeny is an effective strategy to maintain the viral population survival from unfavorable soil microenvironment (Williamson et al., 2007; Abedon, 2011; Williamson et al., 2017a; Trubl et al., 2018a).

4.4.4 The roles of viruses on influencing C and N cycling through the link with functional host

Different vOTU linked to the same *Acidobacteriota*, *Proteobacteria*, and *Verrucomicrobiota* and *Firmicutes* as host been reported in other studies (Emerson et al., 2018; Trubl et al., 2018a). Multiple vOTUs related to *Chthoniobacterales*, within the phylum *Verrucomicrobiota*. The fertilization increased the abundance of *Chthoniobacterales* species. *Chthoniobacterales* have contributed to carbon cycling by decomposing carbohydrates, such as cellulose and xylan (Köberl et al., 2020). The results indicated that *Chthoniobacterales* phage could influence the carbon cycling by control its host. *Nitrososphaeraceae* affiliated with *Thaumarchaeota* phylum are ammonia oxidizer, which plays important roles in soil nitrification (Spang et al., 2010; Tourna et al., 2011; Han et al., 2017). It has been observed that ammonia-oxidizing archaea (AOA) can grow in a wide range of ammonia concentrations (Di et al., 2010; Tourna et al., 2011; Shen et al., 2014)

Above all, the study identified strong and close correlation the link between the virus and its predicted host, and their host can involve in carbon and nitrogen cycling and prophages in the soil. The findings provided the evidence for the soil viruses found in the long-term managed agricultural soil tend to control carbon and nitrogen cycling by infecting the functional host, which consists with the results reported in other soil systems (Emerson et al., 2018; Trubl et al., 2018a; ter Horst et al., 2020).

4.5 Conclusions

The 260 vOTUs including 232 novel viruses and 28 previously reported were identified from through twelve bulk soil metagenomes with 10 Gbp output per library across over 40 years inorganic N fertilization and cover cropping treated soil. Multiple vOTUs at genus or higher level can be potentially linked to the microbial genus level or higher level within same phyla. The microbial host populations were the key C and N cycling taxa including *Acidobacteriota*, *Proteobacteria*, *Verrucomicrobiota*, *Firmicutes*, and ammonia oxidizing archaea (AOA) *Nitrososphaeria*. Long-term inorganic N fertilization has greater effect on influencing viral alpha-diversity, community structure, and positively correlation between viruses and their potential hosts than cover cropping. These findings indicated a close relationship between virus and microbes, virus can modulate the abundance of functional taxa and host to influence soil microbial function in C and N cycling. Although bulk-soil metagenome is an effective approach for detecting both viruses and their host, viromics as a complement on studying viral ecology is suggested in this study.

References

Abbasi, M., Adams, W. 2000. Gaseous N emission during simultaneous nitrification–denitrification associated with mineral N fertilization to a grassland soil under field conditions. Soil Biology and Biochemistry, 32(8-9), 1251-1259.

Abdi, H., Williams, L. J. 2010. Tukey's honestly significant difference (HSD) test. Encyclopedia of research design, 3(1), 1-5.

Abedon, S. T. 2009. Disambiguating bacteriophage pseudolysogeny: an historical analysis of lysogeny, pseudolysogeny, and the phage carrier state. Contemporary trends in bacteriophage research, 285-307.

Abedon, S. T. (2011). Communication among phages, bacteria, and soil environments. In *Biocommunication in soil microorganisms* (pp. 37-65): Springer.

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I. 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. BioScience, 48(11), 921-934.

Acharya, B. S., Dodla, S., Gaston, L. A., Darapuneni, M., Wang, J. J., Sepat, S., Bohara, H. 2019. Winter cover crops effect on soil moisture and soybean growth and yield under different tillage systems. Soil and Tillage Research, 195, 104430.

Acosta-Martínez, V., Burow, G., Zobeck, T., Allen, V. 2010. Soil microbial communities and function in alternative systems to continuous cotton. Soil Science Society of America Journal, 74(4), 1181-1192.

Aczel, M. R. 2019. What is the nitrogen cycle and why is it key to life?

Adriaenssens, E. M., Van Zyl, L., De Maayer, P., Rubagotti, E., Rybicki, E., Tuffin, M., Cowan, D. A. 2015. Metagenomic analysis of the viral community in Namib Desert hypoliths. Environ Microbiol, 17(2), 480-495.

Albrecht, A., Ottow, J., Benckiser, G., Sich, I., Russow, R. 1997. Incomplete denitrification (NO and N2O) from nitrate by Streptomyces violaceoruber and S. nitrosporeus revealed by acetylene inhibition and 15N gas chromatography-quadrupole mass spectrometry analyses. Naturwissenschaften, 84(4), 145-147.

Allen, B., Willner, D., Oechel, W. C., Lipson, D. 2010a. Top-down control of microbial activity and biomass in an Arctic soil ecosystem. Environmental microbiology, 12(3), 642-648.

Allen, B., Willner, D., Oechel, W. C., Lipson, D. J. E. m. 2010b. Top-down control of microbial activity and biomass in an Arctic soil ecosystem. 12(3), 642-648.

Allison, S. D., Martiny, J. B. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences, 105(Supplement 1), 11512-11519.

Alneberg, J., Bjarnason, B. S., de Bruijn, I., Schirmer, M., Quick, J., Ijaz, U. Z., Loman, N. J., Andersson, A. F., Quince, C. 2013. CONCOCT: clustering contigs on coverage and composition. arXiv preprint arXiv:1312.4038.

Anand, M., Baidyanath, K., Dina, N. 2015. Cyanobacterial consortium in the improvement of maize crop. International Journal of Current Microbiology and Applied Sciences, 4(3), 264-274.

Anderson, M. J. 2014. Permutational multivariate analysis of variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online, 1-15.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data.

Armanious, A., Aeppli, M., Jacak, R., Refardt, D., Sigstam, T., Kohn, T., Sander, M. 2016. Viruses at solid–water interfaces: a systematic assessment of interactions driving adsorption. Environmental science & technology, 50(2), 732-743.

Ashelford, K. E., Day, M. J., Fry, J. C. 2003. Elevated abundance of bacteriophage infecting bacteria in soil. Applied and environmental microbiology, 69(1), 285-289.

Ashelford, K. E., Norris, S. J., Fry, J. C., Bailey, M. J., Day, M. J. 2000. Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere. Appl Environ Microbiol, 66(10), 4193-4199.

Averill, C., Waring, B. 2018. Nitrogen limitation of decomposition and decay: How can it occur? Global Change Biology, 24(4), 1417-1427.

Awonaike, K., Kumarasinghe, K., Danso, S. 1990. Nitrogen fixation and yield of cowpea (Vigna unguiculata) as influenced by cultivar and Bradyrhizobium strain. Field Crops Research, 24(3-4), 163-171.

Azam, F., Fenchel, T., Field, J. G., Gray, J., Meyer-Reil, L., Thingstad, F. 1983. The ecological role of water-column microbes in the sea. Marine ecology progress series, 257-263.

Bacon, J. (1968). The Chemical Envir-onments of bacteria in soil. In the Ecology of Soil Bacteria. In: Liverpool University press.

Bardgett, R. D., Shine, A. 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology & Biochemistry, 31(2), 317-321.

Batjes, N. H. 2014. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science, 65(1), 10-21.

Baumann, B., Snozzi, M., Zehnder, A., Van Der Meer, J. R. 1996. Dynamics of denitrification activity of Paracoccus denitrificans in continuous culture during aerobic-anaerobic changes. Journal of bacteriology, 178(15), 4367-4374.

Beier, S., Bertilsson, S. 2013. Bacterial chitin degradation—mechanisms and ecophysiological strategies. Frontiers in Microbiology, 4, 149.

Bending, G. D., Turner, M. K., Jones, J. E. J. S. B., Biochemistry. 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. 34(8), 1073-1082.

Benjamini, Y. 1995. Benjamini and Hochberg methods. Journal of the Royal statistical society: series B (Methodological), 57, 289-300.

Benjamini, Y., Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological), 57(1), 289-300.

Bentler, P. M. 1990. Comparative fit indexes in structural models. Psychological bulletin, 107(2), 238.

Bertozzi Silva, J., Storms, Z., Sauvageau, D. 2016. Host receptors for bacteriophage adsorption. FEMS microbiology letters, 363(4).

Besra, G. S., Khoo, K.-H., McNeil, M. R., Dell, A., Morris, H. R., Brennan, P. J. 1995. A new interpretation of the structure of the mycolyl-arabinogalactan complex of Mycobacterium tuberculosis as revealed through characterization of oligoglycosylalditol fragments by fast-atom bombardment mass spectrometry and 1H nuclear magnetic resonance spectroscopy. Biochemistry, 34(13), 4257-4266.

Bobay, L.-M., Rocha, E. P., Touchon, M. 2013. The adaptation of temperate bacteriophages to their host genomes. Molecular biology and evolution, 30(4), 737-751.

Bolduc, B., Jang, H. B., Doulcier, G., You, Z.-Q., Roux, S., Sullivan, M. B. 2017. vConTACT: an iVirus tool to classify double-stranded DNA viruses that infect Archaea and Bacteria. PeerJ, 5, e3243.

Borrel, G., Colombet, J., Robin, A., Lehours, A.-C., Prangishvili, D., Sime-Ngando, T. 2012. Unexpected and novel putative viruses in the sediments of a deep-dark permanently anoxic freshwater habitat. The ISME journal, 6(11), 2119-2127.

Botton, S., van Heusden, M., Parsons, J. R., Smidt, H., van Straalen, N. 2006. Resilience of microbial systems towards disturbances. Crit Rev Microbiol, 32(2), 101-112.

Breitbart, M., Bonnain, C., Malki, K., Sawaya, N. A. 2018. Phage puppet masters of the marine microbial realm. Nature Microbiology, 3(7), 754-766.

Breitbart, M., Thompson, L. R., Suttle, C. A., Sullivan, M. B. 2007. Exploring the vast diversity of marine viruses. Oceanography, 20(2), 135-139.

Briandet, R., Lacroix-Gueu, P., Renault, M., Lecart, S., Meylheuc, T., Bidnenko, E., Steenkeste, K., Bellon-Fontaine, M.-N., Fontaine-Aupart, M.-P. 2008. Fluorescence correlation spectroscopy to study diffusion and reaction of bacteriophages inside biofilms. Applied and environmental microbiology, 74(7), 2135-2143.

Browne, M. W., Cudeck, R. 1993. Alternative ways of assessing model fit In: Bollen KA, Long JS, editors. Testing structural equation models. Beverly Hills, CA: Sage, 111-135.

Bruns, M. A., Stephen, J. R., Kowalchuk, G. A., Prosser, J. I., Paul, E. A. 1999. Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled, and successional soils. Applied and environmental microbiology, 65(7), 2994-3000.

Bu, R., Ren, T., Lei, M., Liu, B., Li, X., Cong, R., Zhang, Y., Lu, J. 2020. Tillage and straw-returning practices effect on soil dissolved organic matter, aggregate fraction and bacteria community under rice-rice-rapeseed rotation system. Agriculture, Ecosystems & Environment, 287, 106681.

Buckley, D., Schmidt, T. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microbial Ecology, 42(1), 11-21.

Burns, K. N., Bokulich, N. A., Cantu, D., Greenhut, R. F., Kluepfel, D. A., O'Geen, A. T., Strauss, S. L., Steenwerth, K. L. 2016. Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by vineyard management. Soil Biology and Biochemistry, 103, 337-348.

Bushnell, B. 2015. BBMap short-read aligner, and other bioinformatics tools. University of California, Berkeley, CA.

Byrne, B. M. 2013. Structural equation modeling with AMOS: Basic concepts, applications, and programming (2nd ed.): New York: Routledge.

Cardoso, E. J. B. N., Vasconcellos, R. L. F., Bini, D., Miyauchi, M. Y. H., Santos, C. A. d., Alves, P. R. L., Paula, A. M. d., Nakatani, A. S., Pereira, J. d. M., Nogueira, M. A. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? Scientia Agricola, 70(4), 274-289.

Cardoso, N. C., Kana, B. D. 2019. The contribution of the NarB and NarGHI enzymes to nitrate reduction in Mycobacterium smegmatis.

Carlson, C. A., Ingraham, J. L. 1983. Comparison of denitrification by Pseudomonas stutzeri, Pseudomonas aeruginosa, and Paracoccus denitrificans. Applied and environmental microbiology, 45(4), 1247-1253.

Carmines, E. G., McIver, J. P. 1983. An introduction to the analysis of models with unobserved variables. Political Methodology, 51-102.

Chai, Y., Beauregard, P. B., Vlamakis, H., Losick, R., Kolter, R. 2012. Galactose metabolism plays a crucial role in biofilm formation by Bacillus subtilis. MBio, 3(4), e00184-00112.

Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., Parks, D. H. (2020). GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. In: Oxford University Press.

Che, R., Qin, J., Tahmasbian, I., Wang, F., Zhou, S., Xu, Z., Cui, X. 2018. Litter amendment rather than phosphorus can dramatically change inorganic nitrogen pools in a degraded grassland soil by affecting nitrogen-cycling microbes. Soil Biology and Biochemistry, 120, 145-152.

Chen, L., Xun, W., Sun, L., Zhang, N., Shen, Q., Zhang, R. 2014. Effect of different long-term fertilization regimes on the viral community in an agricultural soil of Southern China. European Journal of Soil Biology, 62, 121-126.

Chen, Z., Liu, J., Wu, M., Xie, X., Wu, J., Wei, W. 2012. Differentiated response of denitrifying communities to fertilization regime in paddy soil. Microbial Ecology, 63(2), 446-459.

Cheshire, M. (1985). Carbohydrates in relation to soil fertility. In *Soil organic matter and biological activity* (pp. 263-288): Springer.

Chow, C. E. T., Kim, D. Y., Sachdeva, R., Caron, D. A., Fuhrman, J. A. 2014. Top-down controls on bacterial community structure: microbial network analysis of bacteria, T4-like viruses and protists. The ISME journal, 8(4), 816-829.

Cobián Güemes, A. G., Youle, M., Cantú, V. A., Felts, B., Nulton, J., Rohwer, F. J. A. R. o. V. 2016. Viruses as winners in the game of life. 3, 197-214.

Craine, J. M., Morrow, C., Fierer, N. 2007. Microbial nitrogen limitation increases decomposition. Ecology, 88(8), 2105-2113.

Crummett, L. T., Puxty, R. J., Weihe, C., Marston, M. F., Martiny, J. B. 2016. The genomic content and context of auxiliary metabolic genes in marine cyanomyoviruses. Virology, 499, 219-229.

Cua, L. S., Stein, L. Y. 2014. Characterization of denitrifying activity by the alphaproteobacterium, Sphingomonas wittichii RW1. Frontiers in Microbiology, 5, 404.

Culman, S., Freeman, M., Snapp, S. 2012. Procedure for the determination of permanganate oxidizable carbon. Hickory Corners MI: Kellogg Biological Station.

Dai, Z., Su, W., Chen, H., Barberán, A., Zhao, H., Yu, M., Yu, L., Brookes, P. C., Schadt, C. W., Chang, S. X. 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. Global Change Biology, 24(8), 3452-3461.

Danovaro, R., Dell'Anno, A., Corinaldesi, C., Magagnini, M., Noble, R., Tamburini, C., Weinbauer, M. 2008. Major viral impact on the functioning of benthic deep-sea ecosystems. Nature, 454(7208), 1084-1087.

Datta, D. B., Arden, B., Henning, U. 1977. Major proteins of the Escherichia coli outer cell envelope membrane as bacteriophage receptors. Journal of bacteriology, 131(3), 821-829.

Davey, A., Marchant, H. J. 1983. Seasonal variation in nitrogen fixation by Nostoc commune Vaucher at the Vestfold Hills, Antarctica. Phycologia, 22(4), 377-385.

de Almeida, R. F., Naves, E. R., da Mota, R. P. 2015. Soil quality: Enzymatic activity of soil β-glucosidase. Global Journal of Agricultural Research and Reviews, 3(2), 146-150.

De Corte, D., Sintes, E., Winter, C., Yokokawa, T., Reinthaler, T., Herndl, G. J. 2010. Links between viral and prokaryotic communities throughout the water column in the (sub) tropical Atlantic Ocean. The ISME journal, 4(11), 1431-1442.

De Smet, J., Zimmermann, M., Kogadeeva, M., Ceyssens, P.-J., Vermaelen, W., Blasdel, B., Jang, H. B., Sauer, U., Lavigne, R. 2016. High coverage metabolomics analysis reveals phage-specific alterations to Pseudomonas aeruginosa physiology during infection. The ISME journal, 10(8), 1823-1835.

DeAngelis, K. M., Silver, W. L., Thompson, A. W., Firestone, M. K. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental microbiology, 12(12), 3137-3149.

Di, H. J., Cameron, K. C., Shen, J., Winefield, C. S., O'Callaghan, M., Bowatte, S., He, J. 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS microbiology ecology, 72(3), 386-394.

Dick, R. P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agriculture, Ecosystems & Environment, 40(1-4), 25-36.

Djaman, K., O'Neill, M., Owen, C., Smeal, D., West, M., Begay, D., Allen, S., Koudahe, K., Irmak, S., Lombard, K. J. A. 2018. Long-Term Winter Wheat (Triticum aestivum L.) Seasonal Irrigation Amount, Evapotranspiration, Yield, and Water Productivity under Semiarid Climate. 8(6), 96.

Doane, T. A., Horwáth, W. R. 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical letters, 36(12), 2713-2722.

Drijber, R. A., Doran, J. W., Parkhurst, A. M., Lyon, D. J. S. B., Biochemistry. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. 32(10), 1419-1430.

Du, C., Jiang, S., Jiang, S., Zhou, Y., Zhang, G. 2019. A Bacillus pumilus originated β-N-acetylglucosaminidase for chitin combinatory hydrolysis and exploration of its thermostable mechanism. International journal of biological macromolecules, 132, 1282-1289.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research, 32(5), 1792-1797.

Edlin, G., Lin, L., Bitner, R. 1977. Reproductive fitness of P1, P2, and Mu lysogens of Escherichia coli. Journal of virology, 21(2), 560-564.

Edlin, G., Lin, L., Kudrna, R. 1975. λ lysogens of E. coli reproduce more rapidly than non-lysogens. Nature, 255(5511), 735-737.

Ekschmitt, K., Liu, M., Vetter, S., Fox, O., Wolters, V. J. G. 2005. Strategies used by soil biota to overcome soil organic matter stability—why is dead organic matter left over in the soil? Geoderma, 128(1-2), 167-176.

Emerson, J. B., Roux, S., Brum, J. R., Bolduc, B., Woodcroft, B. J., Jang, H. B., Singleton, C. M., Solden, L. M., Naas, A. E., Boyd, J. A. 2018. Host-linked soil viral ecology along a permafrost thaw gradient. Nature Microbiology, 3(8), 870-880.

Enav, H., Mandel-Gutfreund, Y., Béjà, O. 2014. Comparative metagenomic analyses reveal viral-induced shifts of host metabolism towards nucleotide biosynthesis. Microbiome, 2(1), 1-12.

Etemadi, F., Hashemi, M., Zandvakili, O., Dolatabadian, A., Sadeghpour, A. 2018. Nitrogen contribution from winter-killed faba bean cover crop to spring-sown sweet corn in conventional and no-till systems. Agronomy Journal, 110(2), 455-462.

Evans, J. T., Denef, V. J. 2020. To dereplicate or not to dereplicate? Msphere, 5(3).

Fanin, N., Fromin, N., Bertrand, I. 2016. Functional breadth and home-field advantage generate functional differences among soil microbial decomposers. Ecology, 97(4), 1023-1037.

Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., Owens, S., Gilbert, J. A., Wall, D. H., Caporaso, J. G. J. P. o. t. N. A. o. S. 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences, 109(52), 21390-21395.

Fierer, N., Schimel, J. P. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry, 34(6), 777-787.

Finney, D. M., Buyer, J. S., Kaye, J. P. 2017. Living cover crops have immediate impacts on soil microbial community structure and function. Journal of Soil and Water Conservation, 72(4), 361-373.

Fornelos, N., Browning, D. F., Pavlin, A., Podlesek, Z., Hodnik, V., Salas, M., Butala, M. 2018. Lytic gene expression in the temperate bacteriophage GIL01 is activated by a phage-encoded LexA homologue. Nucleic acids research, 46(18), 9432-9443.

Fuhrman, J. (1992). Bacterioplankton roles in cycling of organic matter: the microbial food web. In *Primary productivity and biogeochemical cycles in the sea* (pp. 361-383): Springer.

Fuhrman, J. A. 1999a. Marine viruses and their biogeochemical and ecological effects. Nature, 399(6736), 541-548.

Fuhrman, J. A. J. N. 1999b. Marine viruses and their biogeochemical and ecological effects. 399(6736), 541.

Fuhrmann, J., Davey, C., Wollum, A. 1986. Desiccation tolerance of clover rhizobia in sterile soils. Soil Science Society of America Journal, 50(3), 639-644.

Fukami, J., Cerezini, P., Hungria, M. 2018. Azospirillum: benefits that go far beyond biological nitrogen fixation. AMB Express, 8(1), 1-12.

Gabiatti, N., Yu, P., Mathieu, J., Lu, G. W., Wang, X., Zhang, H., Soares, H. M., Alvarez, P. J. 2018. Bacterial endospores as phage genome carriers and protective shells. Applied and environmental microbiology, 84(18).

Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B., Cosby, B. J. 2003. The nitrogen cascade. BioScience, 53(4), 341-356.

Gazitúa, M. C., Vik, D. R., Roux, S., Gregory, A. C., Bolduc, B., Widner, B., Mulholland, M. R., Hallam, S. J., Ulloa, O., Sullivan, M. B. 2020. Potential virus-mediated nitrogen cycling in oxygen-depleted oceanic waters. The ISME journal, 1-18.

Gee, C. S., Pfeffer, J. T., Suidan, M. T. 1990. Nitrosomonas and Nitrobacter interactions in biological nitrification. Journal of environmental Engineering, 116(1), 4-17.

Geisseler, D., Scow, K. M. 2014. Long-term effects of mineral fertilizers on soil microorganisms–A review. Soil Biology and Biochemistry, 75, 54-63.

Gerba, C. P. 1984. Applied and theoretical aspects of virus adsorption to surfaces. Advances in applied microbiology, 30, 133-168.

Ghosh, D., Roy, K., Williamson, K. E., Srinivasiah, S., Wommack, K. E., Radosevich, M. J. A., microbiology, e. 2009a. Acyl-homoserine lactones can induce virus production in lysogenic bacteria: an alternative paradigm for prophage induction. 75(22), 7142-7152.

Ghosh, D., Roy, K., Williamson, K. E., Srinivasiah, S., Wommack, K. E., Radosevich, M. J. A., microbiology, e. 2009b. Acyl-homoserine lactones can induce virus production in lysogenic bacteria: an alternative paradigm for prophage induction. Applied and environmental microbiology, 75(22), 7142-7152.

Gliessman, S., Tittonell, P. (2015). Agroecology for food security and nutrition. In: Taylor & Francis.

Goyal, S. M., Gerba, C. P. 1979. Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. Applied and environmental microbiology, 38(2), 241-247.

Graham, E. B., Paez-Espino, D., Brislawn, C., Neches, R. Y., Hofmockel, K. S., Wu, R., Kyrpides, N. C., Jansson, J. K., McDermott, J. E. 2019. Untapped viral diversity in global soil metagenomes. bioRxiv, 583997.

Grange, L. J., Smith, C. R. 2013. Megafaunal communities in rapidly warming fjords along the West Antarctic Peninsula: hotspots of abundance and beta diversity. PLoS One, 8(12), e77917.

Griffiths, R. I., Manefield, M., Ostle, N., McNamara, N., O'Donnell, A. G., Bailey, M. J., Whiteley, A. S. 2004. 13CO2 pulse labelling of plants in tandem with stable isotope probing: methodological considerations for examining microbial function in the rhizosphere. J Microbiol Methods, 58(1), 119-129.

Guemes, A. G. C., Youle, M., Cantu, V. A., Felts, B., Nulton, J., Rohwer, F. 2016. Viruses as Winners in the Game of Life. Annual review of virology, 3, 197-214.

Hagedorn, F., Spinnler, D., Siegwolf, R. 2003. Increased N deposition retards mineralization of old soil organic matter. Soil Biology and Biochemistry, 35(12), 1683-1692.

Han, J., Shi, J., Zeng, L., Xu, J., Wu, L. 2017. Impacts of continuous excessive fertilization on soil potential nitrification activity and nitrifying microbial community dynamics in greenhouse system. Journal of Soils and Sediments, 17(2), 471-480.

Handelsman, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev, 68(4), 669-685.

Heather, J. M., Chain, B. 2016. The sequence of sequencers: The history of sequencing DNA. Genomics, 107(1), 1-8.

Helmi, K., Skraber, S., Gantzer, C., Willame, R., Hoffmann, L., Cauchie, H.-M. 2008. Interactions of Cryptosporidium parvum, Giardia lamblia, vaccinal poliovirus type 1, and bacteriophages ΦX174 and MS2 with a drinking water biofilm and a wastewater biofilm. Applied and environmental microbiology, 74(7), 2079-2088.

Helton, R. R., Wommack, K. E. 2009. Seasonal dynamics and metagenomic characterization of estuarine viriobenthos assemblages by randomly amplified polymorphic DNA PCR. Appl Environ Microbiol, 75(8), 2259-2265.

Hennes, K. P., Suttle, C. A., Chan, A. M. 1995. Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. Applied and environmental microbiology, 61(10), 3623-3627.

Heo, H., Kwon, M., Song, B., Yoon, S. 2020. Involvement of NO3− in ecophysiological regulation of dissimilatory nitrate/nitrite reduction to ammonium (DNRA) is implied by physiological characterization of soil DNRA bacteria isolated via a colorimetric screening method. Applied and environmental microbiology, 86(17), e01054-01020.

Hewson, I., Fuhrman, J. A. 2003. Viriobenthos production and virioplankton sorptive scavenging by suspended sediment particles in coastal and pelagic waters. Microbial Ecology, 46(3), 337-347.

Hobbs, P. R., Sayre, K., Gupta, R. 2008. The role of conservation agriculture in sustainable agriculture. Philosophical Transactions of the Royal Society B: Biological Sciences, 363(1491), 543-555.

Holden, H. M., Rayment, I., Thoden, J. B. 2003. Structure and function of enzymes of the Leloir pathway for galactose metabolism. Journal of Biological Chemistry, 278(45), 43885-43888.

Holland, J. M. 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agriculture, Ecosystems & Environment, 103(1), 1-25.

Homyak, P. M., Allison, S. D., Huxman, T. E., Goulden, M. L., Treseder, K. K. 2017. Effects of drought manipulation on soil nitrogen cycling: A meta-analysis. Journal of Geophysical Research: Biogeosciences, 122(12), 3260-3272.

Hu, J., Jin, V. L., Konkel, J. Y., Schaeffer, S. M., Schneider, L. G., DeBruyn, J. M. 2021a. Soil Health Management Enhances Microbial Nitrogen Cycling Capacity and Activity. Msphere, 6(1), e01237-01220.

Hu, J., Richwine, J. D., Keyser, P. D., Li, L., Yao, F., Jagadamma, S., DeBruyn, J. M. 2021b. Nitrogen Fertilization and Native C4 Grass Species Alter Abundance, Activity, and Diversity of Soil Diazotrophic Communities. Frontiers in Microbiology, 12.

Huang, W., Hall, S. J. 2017. Elevated moisture stimulates carbon loss from mineral soils by releasing protected organic matter. Nature Communications, 8(1), 1-10.

Huang, X., Jiao, N., Zhang, R. 2021. The genomic content and context of auxiliary metabolic genes in roseophages. Environmental microbiology.

Hurek, T., Reinhold-Hurek, B. 2003. Azoarcus sp. strain BH72 as a model for nitrogen-fixing grass endophytes. Journal of biotechnology, 106(2-3), 169-178.

Hurst, C. J., Gerba, C. P., Cech, I. 1980a. Effects of environmental variables and soil characteristics on virus survival in soil. Appl Environ Microbiol, 40(6), 1067-1079.

Hurst, C. J., Gerba, C. P., Cech, I. 1980b. Effects of environmental variables and soil characteristics on virus survival in soil. Applied and environmental microbiology, 40(6), 1067-1079.

Hurwitz, B. L., Hallam, S. J., Sullivan, M. B. 2013. Metabolic reprogramming by viruses in the sunlit and dark ocean. Genome biology, 14(11), 1-14.

Hyatt, D., LoCascio, P. F., Hauser, L. J., Uberbacher, E. C. 2012. Gene and translation initiation site prediction in metagenomic sequences. Bioinformatics, 28(17), 2223-2230.

Isobe, K., Ohte, N. 2014. Ecological perspectives on microbes involved in N-cycling. Microbes and environments, ME13159.

Ivanova, A. A., Zhelezova, A. D., Chernov, T. I., Dedysh, S. N. 2020. Linking ecology and systematics of acidobacteria: Distinct habitat preferences of the Acidobacteriia and Blastocatellia in tundra soils. PLoS One, 15(3), e0230157.

Jackson, L., Calderon, F., Steenwerth, K., Scow, K., Rolston, D. 2003. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. Geoderma, 114(3-4), 305-317.

Jain, C., Rodriguez-R, L. M., Phillippy, A. M., Konstantinidis, K. T., Aluru, S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nature Communications, 9(1), 1-8.

Janda, J. M., Abbott, S. L. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol, 45(9), 2761-2764.

Jansson, J. K., Hofmockel, K. S. 2018. The soil microbiome—from metagenomics to metaphenomics. Current opinion in microbiology, 43, 162-168.

Jian, S., Li, J., Chen, J., Wang, G., Mayes, M. A., Dzantor, K. E., Hui, D., Luo, Y. 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biology and Biochemistry, 101, 32-43.

Jiménez-Bueno, N., Valenzuela-Encinas, C., Marsch, R., Ortiz-Gutiérrez, D., Verhulst, N., Govaerts, B., Dendooven, L., Navarro-Noya, Y. 2016. Bacterial indicator taxa in soils under different long-term agricultural management. Journal of applied microbiology, 120(4), 921-933.

Jover, L. F., Effler, T. C., Buchan, A., Wilhelm, S. W., Weitz, J. S. 2014. The elemental composition of virus particles: implications for marine biogeochemical cycles. Nature Reviews Microbiology, 12(7), 519-528.

Kamasaka, H., Sugimoto, K., Takata, H., Nishimura, T., Kuriki, T. 2002. Bacillus stearothermophilus neopullulanase selective hydrolysis of amylose to maltose in the presence of amylopectin. Applied and environmental microbiology, 68(4), 1658-1664.

Kang, D. D., Li, F., Kirton, E., Thomas, A., Egan, R., An, H., Wang, Z. 2019. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ, 7, e7359.

Karlin, S., Brendel, V. 1988. Charge configurations in viral proteins. Proceedings of the National Academy of Sciences, 85(24), 9396-9400.

Kenst, A. B., Perfect, E., Wilhelm, S. W., Zhuang, J., McCarthy, J. F., McKay, L. D. 2008. Virus transport during infiltration of a wetting front into initially unsaturated sand columns. Environmental science & technology, 42(4), 1102-1108.

Khan, A. 1996. Influence of tillage on soil aeration. Journal of agronomy and crop science, 177(4), 253-259.

Kibblewhite, M., Ritz, K., Swift, M. J. P. T. o. t. R. S. o. L. B. B. S. 2008. Soil health in agricultural systems. 363(1492), 685-701.

Kieft, K., Zhou, Z., Anantharaman, K. 2020. VIBRANT: automated recovery, annotation and curation of microbial viruses, and evaluation of viral community function from genomic sequences. Microbiome, 8(1), 1-23.

Kikuchi, J., Komatsu, T. (2017). Polysaccharides as major carbon sources in environmental biodiversity. In *NMR in Glycoscience and Glycotechnology* (pp. 369-395).

Kim, B. R., Shin, J., Guevarra, R. B., Lee, J. H., Kim, D. W., Seol, K. H., Lee, J. H., Kim, H. B., Isaacson, R. E. 2017. Deciphering Diversity Indices for a Better Understanding of Microbial Communities. Journal of Microbiology and Biotechnology, 27(12), 2089-2093.

Kim, H.-J., Kang, S. Y., Park, J. J., Kim, P. 2011. Novel activity of UDP-galactose-4-epimerase for free monosaccharide and activity improvement by active site-saturation mutagenesis. Applied biochemistry and biotechnology, 163(3), 444-451.

Kimura, M., Jia, Z., Jun, Nakayama, N., Asakawa, S. 2008a. Ecology of viruses in soils: past, present and future perspectives. Soil Science and Plant Nutrition, 54(1), 1-32.

Kimura, M., Jia, Z.-J., Nakayama, N., Asakawa, S. J. S. S., Nutrition, P. 2008b. Ecology of viruses in soils: past, present and future perspectives. 54(1), 1-32.

Kladivko, E. J. 2001. Tillage systems and soil ecology. Soil & Tillage Research, 61(1-2), 61-76.

Kladivko, E. J. J. S., Research, T. 2001. Tillage systems and soil ecology. 61(1-2), 61-76.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F. O. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic acids research, 41(1), e1-e1.

Knecht, L. E., Veljkovic, M., Fieseler, L. 2020. Diversity and function of phage encoded depolymerases. Frontiers in Microbiology, 10, 2949.

Köberl, M., Wagner, P., Müller, H., Matzer, R., Unterfrauner, H., Cernava, T., Berg, G. 2020. Unraveling the complexity of soil microbiomes in a large-scale study subjected to different agricultural management in Styria. Frontiers in Microbiology, 11, 1052.

Koonin, E. V., Senkevich, T. G., Dolja, V. V. 2006. The ancient Virus World and evolution of cells. Biol Direct, 1, 29.

Kostka, J. E., Green, S. J., Rishishwar, L., Prakash, O., Katz, L. S., Mariño-Ramírez, L., Jordan, I. K., Munk, C., Ivanova, N., Mikhailova, N. (2012). Genome sequences for six Rhodanobacter strains, isolated from soils and the terrestrial subsurface, with variable denitrification capabilities. In: Am Soc Microbiol.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., Schloss, P. D. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology, 79(17), 5112-5120.

Kraft, B., Strous, M., Tegetmeyer, H. E. 2011. Microbial nitrate respiration–genes, enzymes and environmental distribution. Journal of biotechnology, 155(1), 104-117.

Krispin, O., Allmansberger, R. 1998. The Bacillus subtilis galE gene is essential in the presence of glucose and galactose. Journal of bacteriology, 180(8), 2265-2270.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, 35(6), 1547-1549.

Kuzyakov, Y., Mason-Jones, K. 2018. Viruses in soil: Nano-scale undead drivers of microbial life, biogeochemical turnover and ecosystem functions. Soil Biology & Biochemistry, 127, 305-317.

Lacroix-Gueu, P., Briandet, R., Lévêque-Fort, S., Bellon-Fontaine, M.-N., Fontaine-Aupart, M.-P. 2005. In situ measurements of viral particles diffusion inside mucoid biofilms. Comptes rendus biologies, 328(12), 1065-1072.

Lance, J., Gerba, C. P. 1984. Effect of ionic composition of suspending solution on virus adsorption by a soil column. Applied and environmental microbiology, 47(3), 484-488.

Lauber, C. L., Ramirez, K. S., Aanderud, Z., Lennon, J., Fierer, N. 2013. Temporal variability in soil microbial communities across land-use types. The ISME journal, 7(8), 1641-1650.

Le, S. Q., Gascuel, O. 2008. An improved general amino acid replacement matrix. Molecular biology and evolution, 25(7), 1307-1320.

LeBauer, D. S., Treseder, K. K. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology, 89(2), 371-379.

Legrand, F., Picot, A., Cobo-Díaz, J. F., Carof, M., Chen, W., Le Floch, G. 2018. Effect of tillage and static abiotic soil properties on microbial diversity. Applied Soil Ecology, 132, 135-145.

Li, D., Liu, C.-M., Luo, R., Sadakane, K., Lam, T.-W. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics, 31(10), 1674-1676.

Li, D., Luo, R., Liu, C.-M., Leung, C.-M., Ting, H.-F., Sadakane, K., Yamashita, H., Lam, T.-W. 2016. MEGAHIT v1. 0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods, 102, 3-11.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics, 25(16), 2078-2079.

Li, H., Zhang, Y., Yang, S., Wang, Z., Feng, X., Liu, H., Jiang, Y. 2019a. Variations in soil bacterial taxonomic profiles and putative functions in response to straw incorporation combined with N fertilization during the maize growing season. Agriculture, Ecosystems & Environment, 283, 106578.

Li, L., Konkel, J., Jin, V. L., Schaeffer, S. M. 2021. Conservation management improves agroecosystem function and resilience of soil nitrogen cycling in response to seasonal changes in climate. Science of the total environment, 779, 146457.

Li, L., Wilson, C. B., He, H., Zhang, X., Zhou, F., Schaeffer, S. M. 2019b. Physical, biochemical, and microbial controls on amino sugar accumulation in soils under long-term cover cropping and no-tillage farming. Soil Biology and Biochemistry, 135, 369-378.

Li, W., Godzik, A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics, 22(13), 1658-1659.

Li, W., Jaroszewski, L., Godzik, A. 2002. Tolerating some redundancy significantly speeds up clustering of large protein databases. Bioinformatics, 18(1), 77-82.

Liang, C., Schimel, J. P., Jastrow, J. D. 2017a. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology, 2(8), 1-6.

Liang, C., Schimel, J. P., Jastrow, J. D. J. N. M. 2017b. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology, 2(8), 17105.

Liang, X., Zhuang, J., Loffler, F. E., Zhang, Y., DeBruyn, J. M., Wilhelm, S. W., Schaeffer, S. M., Radosevich, M. 2019. Viral and bacterial community responses to stimulated Fe(III)-bioreduction during simulated subsurface bioremediation. Environ Microbiol, 21(6), 2043-2055.

Lienhard, P., Tivet, F., Chabanne, A., Dequiedt, S., Lelièvre, M., Sayphoummie, S., Leudphanane, B., Prévost-Bouré, N. C., Séguy, L., Maron, P.-A. J. A. f. s. d. 2013. No-till and cover crops shift soil microbial abundance and diversity in Laos tropical grasslands. 33(2), 375-384.

Linderoth, N. A., Ziermann, R., Haggård-Ljungquist, E., Christie, G. E., Calendar, R. 1991. Nucleotide sequence of the DNA packaging and capsid synthesis geges of bacteriophage P2. Nucleic acids research, 19(25), 7207-7214.

Linn, D., Doran, J. J. S. S. S. o. A. J. 1984a. Aerobic and Anaerobic Microbial Populations in No-till and Plowed Soils 48(4), 794-799.

Linn, D., Doran, J. J. S. S. S. o. A. J. 1984b. Aerobic and Anaerobic Microbial Populations in No-till and Plowed Soils 1. 48(4), 794-799.

Linn, D. M., Doran, J. W. 1984c. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal, 48(6), 1267-1272.

Liu, E., Teclemariam, S. G., Yan, C., Yu, J., Gu, R., Liu, S., He, W., Liu, Q. 2014. Long-term effects of no-tillage management practice on soil organic carbon and its fractions in the northern China. Geoderma, 213, 379-384.

Liu, S., Coyne, M., Grove, J., Flythe, M. 2018. Nitrogen, season, and tillage management influence ammonia oxidizing bacterial communities in long-term maize. Applied Soil Ecology, 129, 98-106.

Liu, X., Li, Q., Li, Y., Guan, G., Chen, S. 2019. Paenibacillus strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. PeerJ, 7, e7445.

Liu, X., Li, Y., Ren, X., Chen, B., Zhang, Y., Shen, C., Wang, F., Wu, D. 2020. Long-term greenhouse cucumber production alters soil bacterial community structure. Journal of Soil Science and Plant Nutrition, 20(2), 306-321.

Loeppmann, S., Semenov, M., Blagodatskaya, E., Kuzyakov, Y. 2016. Substrate quality affects microbial-and enzyme activities in rooted soil. Journal of Plant Nutrition and Soil Science, 179(1), 39-47.

Logsdon, S. D., Karlen, D. L. 2004. Bulk density as a soil quality indicator during conversion to no-tillage. Soil and Tillage Research, 78(2), 143-149.

Lokareddy, R. K., Sankhala, R. S., Roy, A., Afonine, P. V., Motwani, T., Teschke, C. M., Parent, K. N., Cingolani, G. 2017. Portal protein functions akin to a DNA-sensor that couples genome-packaging to icosahedral capsid maturation. Nature Communications, 8(1), 1-11.

Love, M. I., Huber, W., Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology, 15(12), 1-21.

Lucarelli, G., Galleggiante, V., Rutigliano, M., Sanguedolce, F., Cagiano, S., Bufo, P., Lastilla, G., Maiorano, E., Ribatti, D., Giglio, A. 2015. Metabolomic profile of glycolysis and the pentose phosphate pathway identifies the central role of glucose-6-phosphate dehydrogenase in clear cell-renal cell carcinoma. Oncotarget, 6(15), 13371.

Magill, A. H., Aber, J. D. 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant and Soil, 203(2), 301-311.

Mäki, M., Renkonen, R. 2004. Biosynthesis of 6-deoxyhexose glycans in bacteria. Glycobiology, 14(3), 1R-15R.

Mann, N. H. J. P. b. 2005. The third age of phage. 3(5), e182.

Marschner, P., Kandeler, E., Marschner, B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry, 35(3), 453-461.

Marsh, P., Wellington, E. 1994. Phage-host interactions in soil. FEMS microbiology ecology, 15(1-2), 99-107.

Martin, J. P. 1971. Decomposition and binding action of polysaccharides in soil. Soil Biology and Biochemistry, 3(1), 33-41.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal, 17(1), 10-12.

Martínez-García, L. B., Korthals, G., Brussaard, L., Jørgensen, H. B., De Deyn, G. B. 2018. Organic management and cover crop species steer soil microbial community structure

and functionality along with soil organic matter properties. Agriculture, Ecosystems & Environment, 263, 7-17.

Mbuthia, L. W. 2014. Long-Term Impacts of Tillage, Cover Crops, and Nitrogen Rates on Microbial Community Dynamics and Soil Quality Parameters under Continuous Cotton Production in West Tennessee.

Mbuthia, L. W., Acosta-Martinez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N. 2015a. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biology & Biochemistry, 89, 24-34.

Mbuthia, L. W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N. 2015b. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biology and Biochemistry, 89, 24-34.

McGinnis, S., Madden, T. L. 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic acids research, 32(suppl_2), W20-W25.

Meliani, A., Bensoltane, A., Mederbel, K. J. A. J. o. P. N., Technology, F. 2012. Microbial diversity and abundance in soil: related to plant and soil type. 2(1), 10-18.

Menouni, R., Hutinet, G., Petit, M.-A., Ansaldi, M. 2015. Bacterial genome remodeling through bacteriophage recombination. FEMS microbiology letters, 362(1), 1-10.

Middelboe, M. 2000. Bacterial growth rate and marine virus–host dynamics. Microbial Ecology, 40(2), 114-124.

Mikheenko, A., Prjibelski, A., Saveliev, V., Antipov, D., Gurevich, A. 2018. Versatile genome assembly evaluation with QUAST-LG. Bioinformatics, 34(13), i142-i150.

Milani, C., Casey, E., Lugli, G. A., Moore, R., Kaczorowska, J., Feehily, C., Mangifesta, M., Mancabelli, L., Duranti, S., Turroni, F. 2018. Tracing mother-infant transmission of bacteriophages by means of a novel analytical tool for shotgun metagenomic datasets: METAnnotatorX. Microbiome, 6(1), 1-16.

Moorhead, D. L., Sinsabaugh, R. L. 2006. A theoretical model of litter decay and microbial interaction. Ecological monographs, 76(2), 151-174.

Mühling, M., Fuller, N. J., Millard, A., Somerfield, P. J., Marie, D., Wilson, W. H., Scanlan, D. J., Post, A. F., Joint, I., Mann, N. H. J. E. m. 2005. Genetic diversity of marine Synechococcus and co-occurring cyanophage communities: evidence for viral control of phytoplankton. 7(4), 499-508.

Nakamura, T., Maeda, Y., Tanoue, N., Makita, T., Kato, M., Kobayashi, T. 2006. Expression profile of amylolytic genes in Aspergillus nidulans. Biosci Biotechnol Biochem, 70(10), 2363-2370.

Neff, J. C., Townsend, A. R., Gleixner, G., Lehman, S. J., Turnbull, J., Bowman, W. D. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature, 419(6910), 915-917.

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., Setlow, P. 2000. Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiology and molecular biology reviews, 64(3), 548-572.

Norton, J., Ouyang, Y. 2019. Controls and adaptive management of nitrification in agricultural soils. Frontiers in Microbiology, 10, 1931.

Norton, J. M., Stark, J. M. 2011. Regulation and measurement of nitrification in terrestrial systems. Methods in enzymology, 486, 343-368.

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic acids research, 44(D1), D733-D745.

Obeng, N., Pratama, A. A., van Elsas, J. D. 2016. The significance of mutualistic phages for bacterial ecology and evolution. Trends in microbiology, 24(6), 440-449.

Olm, M. R., Brown, C. T., Brooks, B., Banfield, J. F. 2017. dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through de-replication. The ISME journal, 11(12), 2864-2868.

Orwin, K., Dickie, I., Holdaway, R., Wood, J. 2018. A comparison of the ability of PLFA and 16S rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions. Soil Biology and Biochemistry, 117, 27-35.

Ouyang, Y., Evans, S. E., Friesen, M. L., Tiemann, L. K. 2018. Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: a meta-analysis of field studies. Soil Biology and Biochemistry, 127, 71-78.

Paez-Espino, D., Eloe-Fadrosh, E. A., Pavlopoulos, G. A., Thomas, A. D., Huntemann, M., Mikhailova, N., Rubin, E., Ivanova, N. N., Kyrpides, N. C. 2016. Uncovering Earth's virome. Nature, 536(7617), 425-430.

Pagan, J., Child, J., Scowcroft, W., Gibson, A. 1975. Nitrogen fixation by Rhizobium cultured on a defined medium. Nature, 256(5516), 406-407.

Pajares, S., Bohannan, B. J. 2016. Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. Frontiers in Microbiology, 7, 1045.

Paladini, A. C., Leloir, L. 1952. Studies on uridine-diphosphate-glucose. Biochemical Journal, 51(3), 426.

Pan, F., Jackson, M., Ma, Y., McNeil, M. 2001. Cell wall core galactofuran synthesis is essential for growth of mycobacteria. Journal of bacteriology, 183(13), 3991-3998.

Pandey, A., Suter, H., He, J.-Z., Hu, H.-W., Chen, D. 2019. Dissimilatory nitrate reduction to ammonium dominates nitrate reduction in long-term low nitrogen fertilized rice paddies. Soil Biology and Biochemistry, 131, 149-156.

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., Tyson, G. W. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome research, 25(7), 1043-1055.

Parks, D. H., Rinke, C., Chuvochina, M., Chaumeil, P.-A., Woodcroft, B. J., Evans, P. N., Hugenholtz, P., Tyson, G. W. 2017. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. Nature Microbiology, 2(11), 1533-1542.

Pastorelli, R., Vignozzi, N., Landi, S., Piccolo, R., Orsini, R., Seddaiu, G., Roggero, P. P., Pagliai, M. 2013. Consequences on macroporosity and bacterial diversity of adopting a no-tillage farming system in a clayish soil of Central Italy. Soil Biology and Biochemistry, 66, 78-93.

Paul, J. H. 2008. Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? The ISME journal, 2(6), 579-589.

Paustian, K., Larson, E., Kent, J., Marx, E., Swan, A. 2019. Soil C sequestration as a biological negative emission strategy. Frontiers in Climate, 1, 8.

Peoples, M. B., Swan, A. D., Goward, L., Kirkegaard, J. A., Hunt, J. R., Li, G. D., Schwenke, G. D., Herridge, D. F., Moodie, M., Wilhelm, N. 2017. Soil mineral nitrogen benefits

derived from legumes and comparisons of the apparent recovery of legume or fertiliser nitrogen by wheat. Soil Research, 55(6), 600-615.

Pettersson, M., Baath, E. 2003. Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. FEMS microbiology ecology, 45(1), 13-21.

Philippot, L., Hallin, S., Schloter, M. 2007. Ecology of denitrifying prokaryotes in agricultural soil. Advances in Agronomy, 96, 249-305.

Pichinoty, F., Bigliardi-Rouvier, J., Mandel, M., Greenway, B., Méténier, G., Garcia, J.-L. 1976. The isolation and properties of a denitrifying bacterium of the genus Flavobacterium. Antonie Van Leeuwenhoek, 42(3), 349-354.

Postma, J., Van Veen, J., Walter, S. 1989. Influence of different initial soil moisture contents on the distribution and population dynamics of introduced Rhizobium leguminosarum biovar trifolii. Soil Biology and Biochemistry, 21(3), 437-442.

Powelson, D. K., Simpson, J. R., Gerba, C. P. 1991. Effects of organic matter on virus transport in unsaturated flow. Applied and environmental microbiology, 57(8), 2192-2196.

Prasad, S., Malav, L. C., Choudhary, J., Kannojiya, S., Kundu, M., Kumar, S., Yadav, A. N. (2021). Soil microbiomes for healthy nutrient recycling. In *Current Trends in Microbial Biotechnology for Sustainable Agriculture* (pp. 1-21): Springer.

Prescott, C. E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry, 101(1), 133-149.

Price, M. N., Dehal, P. S., Arkin, A. P. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Molecular biology and evolution, 26(7), 1641-1650.

Quinlan, A. R., Hall, I. M. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics, 26(6), 841-842.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., Fierer, N. 2010a. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology, 91(12), 3463-3470.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., Fierer, N. 2010b. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology, 91(12), 3463-3470.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., Fierer, N. J. E. 2010c. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. 91(12), 3463-3470.

Rasmann, S., Kollner, T. G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., Turlings, T. C. J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature, 434(7034), 732-737.

Raun, W., Johnson, G., Phillips, S., Westerman, R. 1998. Effect of long-term N fertilization on soil organic C and total N in continuous wheat under conventional tillage in Oklahoma. Soil and Tillage Research, 47(3-4), 323-330.

Raynaud, X., Nunan, N. 2014. Spatial ecology of bacteria at the microscale in soil. PLoS One, 9(1), e87217.

Reanney, D., Marsh, S. 1973. The ecology of viruses attacking Bacillus stearothermophilus in soil. Soil Biology Biochemistry, 5(4), 399-408.

Ren, J., Song, K., Deng, C., Ahlgren, N., Fuhrman, J., Li, Y., Xie, X., Sun, F. 2018. Identifying viruses from metagenomic data by deep learning. arXiv preprint arXiv: 180607810.

Rhine, E. D., Mulvaney, R. L., Pratt, E. J., Sims, G. K. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Science Society of America Journal, 62(2), 473-480.

Rice, S. A., Tan, C. H., Mikkelsen, P. J., Kung, V., Woo, J., Tay, M., Hauser, A., McDougald, D., Webb, J. S., Kjelleberg, S. 2009. The biofilm life cycle and virulence of Pseudomonas aeruginosa are dependent on a filamentous prophage. The ISME journal, 3(3), 271-282.

Roberts, P., Bol, R., Jones, D. L. 2007. Free amino sugar reactions in soil in relation to soil carbon and nitrogen cycling. Soil Biology and Biochemistry, 39(12), 3081-3092.

Roesch, L. F., Fulthorpe, R. R., Riva, A., Casella, G., Hadwin, A. K., Kent, A. D., Daroub, S. H., Camargo, F. A., Farmerie, W. G., Triplett, E. W. J. T. I. j. 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. 1(4), 283.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F. J. P. 2016. VSEARCH: a versatile open source tool for metagenomics. 4, e2584.

Romdhane, S., Spor, A., Busset, H., Falchetto, L., Martin, J., Bizouard, F., Bru, D., Breuil, M. C., Philippot, L., Cordeau, S. 2019. Cover Crop Management Practices Rather Than Composition of Cover Crop Mixtures Affect Bacterial Communities in No-Till Agroecosystems. Frontiers in Microbiology, 10.

Rong, Q., Cai, Y., Chen, B., Yue, W., Tan, Q. 2017. An enhanced export coefficient based optimization model for supporting agricultural nonpoint source pollution mitigation under uncertainty. Science of the total environment, 580, 1351-1362.

Rousk, J., Baath, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., Fierer, N. 2010a. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J, 4(10), 1340-1351.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., Fierer, N. 2010b. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME journal, 4(10), 1340-1351.

Rousk, J., Brookes, P. C., Baath, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol, 75(6), 1589-1596.

Rousk, J., Brookes, P. C., Bååth, E. 2010c. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology and Biochemistry, 42(6), 926-934.

Roux, S., Brum, J. R., Dutilh, B. E., Sunagawa, S., Duhaime, M. B., Loy, A., Poulos, B. T., Solonenko, N., Lara, E., Poulain, J. 2016. Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. Nature, 537(7622), 689-693.

Roux, S., Enault, F., Hurwitz, B. L., Sullivan, M. B. 2015a. VirSorter: mining viral signal from microbial genomic data. PeerJ, 3, e985.

Roux, S., Hallam, S. J., Woyke, T., Sullivan, M. B. 2015b. Viral dark matter and virus–host interactions resolved from publicly available microbial genomes. Elife, 4, e08490.

Sadie, C. J., Rose, S. H., den Haan, R., van Zyl, W. H. 2011. Co-expression of a cellobiose phosphorylase and lactose permease enables intracellular cellobiose utilisation by Saccharomyces cerevisiae. Appl Microbiol Biotechnol, 90(4), 1373-1380.

Salinas-Garcia, J., Hons, F., Matocha, J., Zuberer, D. 1997. Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization. Biology and Fertility of Soils, 25(2), 182-188.

Sanders, D. A., Staines, A. G., McMahon, S. A., McNeil, M. R., Whitfield, C., Naismith, J. H. 2001. UDP-galactopyranose mutase has a novel structure and mechanism. Nature structural biology, 8(10), 858-863.

Santos-Medellin, C., Zinke, L. A., ter Horst, A. M., Gelardi, D. L., Parikh, S. J., Emerson, J. B. 2020. Viromes outperform total metagenomes in revealing the spatiotemporal patterns of agricultural soil viral communities. bioRxiv.

Schimel, J., Schaeffer, S. M. J. F. i. m. 2012. Microbial control over carbon cycling in soil. 3, 348.

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S. J. S. B., Biochemistry. 2011. Experimental warming effects on the microbial community of a temperate mountain forest soil. 43(7), 1417-1425.

Schloss, P. D. 2020. Reintroducing mothur: 10 years later. Applied and environmental microbiology, 86(2).

Schloss, P. D., Handelsman, J. 2006. Toward a census of bacteria in soil. PLoS Comput Biol, 2(7), e92.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and environmental microbiology, 75(23), 7537-7541.

Schmid, J., Heider, D., Wendel, N. J., Sperl, N., Sieber, V. 2016. Bacterial glycosyltransferases: challenges and opportunities of a highly diverse enzyme class toward tailoring natural products. Frontiers in Microbiology, 7, 182.

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J., Scow, K. 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLoS One, 13(2), e0192953.

Schmitz, K., Protzko, R., Zhang, L., Benz, J. P. 2019. Spotlight on fungal pectin utilization—from phytopathogenicity to molecular recognition and industrial applications. Applied Microbiology and Biotechnology, 103(6), 2507-2524.

Schramm, A., de Beer, D., van den Heuvel, J. C., Ottengraf, S., Amann, R. 1999. Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Applied and environmental microbiology, 65(8), 3690-3696.

Schuch, R., Fischetti, V. A. 2009. The secret life of the anthrax agent Bacillus anthracis: bacteriophage-mediated ecological adaptations. PLoS One, 4(8), e6532.

Sekulovic, O., Fortier, L.-C. 2015. Global transcriptional response of Clostridium difficile carrying the ϕCD38-2 prophage. Applied and environmental microbiology, 81(4), 1364-1374.

Shapiro, S. S., Francia, R. 1972. An approximate analysis of variance test for normality. Journal of the American statistical Association, 67(337), 215-216.

Shapiro, S. S., Wilk, M. B., Chen, H. J. 1968. A comparative study of various tests for normality. Journal of the American statistical Association, 63(324), 1343-1372.

Sharon, I., Battchikova, N., Aro, E.-M., Giglione, C., Meinnel, T., Glaser, F., Pinter, R. Y., Breitbart, M., Rohwer, F., Béja, O. 2011. Comparative metagenomics of microbial traits within oceanic viral communities. The ISME journal, 5(7), 1178-1190.

Sharpton, T. J. 2014. An introduction to the analysis of shotgun metagenomic data. Front Plant Sci, 5, 209.

Shen, J., Zhang, L., He, J. 2014. Contrasting response of nitrification capacity in three agricultural soils to N addition during short-term incubation. Journal of Soils and Sediments, 14(11), 1861-1868.

Shibata, A., Kogure, K., Koike, I., Ohwada, K. J. M. E. P. S. 1997. Formation of submicron colloidal particles from marine bacteria by viral infection. 155, 303-307.

Shinde, S., Zhang, X., Singapuri, S. P., Kalra, I., Liu, X., Morgan-Kiss, R. M., Wang, X. 2020. Glycogen metabolism supports photosynthesis start through the oxidative pentose phosphate pathway in cyanobacteria. Plant physiology, 182(1), 507-517.

Sievers, T., Cook, R. L. 2018. Aboveground and root decomposition of cereal rye and hairy vetch cover crops. Soil Science Society of America Journal, 82(1), 147-155.

Simmons, B. L., Coleman, D. C. J. A. s. e. 2008. Microbial community response to transition from conventional to conservation tillage in cotton fields. 40(3), 518-528.

Skennerton, C. T., Imelfort, M., Tyson, G. W. 2013. Crass: identification and reconstruction of CRISPR from unassembled metagenomic data. Nucleic acids research, 41(10), e105-e105.

Skiba, U., Smith, K. 1993. Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. Soil Biology and Biochemistry, 25(11), 1527-1536.

Smets, W., Leff, J. W., Bradford, M. A., McCulley, R. L., Lebeer, S., Fierer, N. 2016. A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. Soil Biology and Biochemistry, 96, 145-151.

Smith, C. J., Hunt, J. R., Wang, E., Macdonald, B. C., Xing, H., Denmead, O., Zeglin, S., Zhao, Z. 2019. Using fertiliser to maintain soil inorganic nitrogen can increase dryland wheat yield with little environmental cost. Agriculture, Ecosystems & Environment, 286, 106644.

Smith, J. 1966. Some inter-relationships between decomposition of various plant residues and loss of soil organic matter as measured by carbon-14 labelling.

Somoza, J. R., Menon, S., Schmidt, H., Joseph-McCarthy, D., Dessen, A., Stahl, M. L., Somers, W. S., Sullivan, F. X. 2000. Structural and kinetic analysis of Escherichia coli GDP-mannose 4, 6 dehydratase provides insights into the enzyme's catalytic mechanism and regulation by GDP-fucose. Structure, 8(2), 123-135.

Songjuan, G., Weidong, C., Guopeng, Z. 2021. Bacterial communities in paddy soils changed by milk vetch as green manure: A study conducted across six provinces in South China. Pedosphere, 31(4), 521-530.

Spang, A., Hatzenpichler, R., Brochier-Armanet, C., Rattei, T., Tischler, P., Spieck, E., Streit, W., Stahl, D. A., Wagner, M., Schleper, C. 2010. Distinct gene set in two different lineages of ammonia-oxidizing archaea supports the phylum Thaumarchaeota. Trends in microbiology, 18(8), 331-340.

Spieck, E., Sass, K., Keuter, S., Hirschmann, S., Spohn, M., Indenbirken, D., Kop, L. F., Lücker, S., Giaveno, A. 2020. Defining culture conditions for the hidden nitrite-oxidizing bacterium Nitrolancea. Frontiers in Microbiology, 11, 1522.

Srinivasiah, S., Lovett, J., Polson, S., Bhavsar, J., Ghosh, D., Roy, K., Fuhrmann, J. J., Radosevich, M., Wommack, K. E. 2013. Direct assessment of viral diversity in soils by random PCR amplification of polymorphic DNA. Appl Environ Microbiol, 79(18), 5450-5457.

Starr, E. P., Nuccio, E. E., Pett-Ridge, J., Banfield, J. F., Firestone, M. K. 2019. Metatranscriptomic reconstruction reveals RNA viruses with the potential to shape carbon cycling in soil. Proceedings of the National Academy of Sciences, 116(51), 25900-25908.

Stecher, G., Tamura, K., Kumar, S. 2020. Molecular evolutionary genetics analysis (MEGA) for macOS. Molecular biology and evolution, 37(4), 1237-1239.

Strange, R. 1968. Bacterial "glycogen" and survival. Nature, 220(5167), 606-607.

Stres, B., Danevcic, T., Pal, L., Fuka, M. M., Resman, L., Leskovec, S., Hacin, J., Stopar, D., Mahne, I., Mandic-Mulec, I. 2008a. Influence of temperature and soil water content on bacterial, archaeal and denitrifying microbial communities in drained fen grassland soil microcosms. FEMS microbiology ecology, 66(1), 110-122.

Stres, B., Danevčič, T., Pal, L., Fuka, M. M., Resman, L., Leskovec, S., Hacin, J., Stopar, D., Mahne, I., Mandic-Mulec, I. 2008b. Influence of temperature and soil water content on bacterial, archaeal and denitrifying microbial communities in drained fen grassland soil microcosms. FEMS microbiology ecology, 66(1), 110-122.

Sun, S., Badgley, B. D. 2019. Changes in microbial functional genes within the soil metagenome during forest ecosystem restoration. Soil Biology and Biochemistry, 135, 163-172.

Sun, Z., Lv, Y., Liu, Y., Ren, R. 2016. Removal of nitrogen by heterotrophic nitrification-aerobic denitrification of a novel metal resistant bacterium Cupriavidus sp. S1. Bioresource technology, 220, 142-150.

Suttle, C. A. 2005. Viruses in the sea. Nature, 437(7057), 356-361.

Suzuki, E., Ohkawa, H., Moriya, K., Matsubara, T., Nagaike, Y., Iwasaki, I., Fujiwara, S., Tsuzuki, M., Nakamura, Y. 2010. Carbohydrate metabolism in mutants of the cyanobacterium Synechococcus elongatus PCC 7942 defective in glycogen synthesis. Applied and environmental microbiology, 76(10), 3153-3159.

Tanaka, J. S., Huba, G. J. 1985. A fit index for covariance structure models under arbitrary GLS estimation. British Journal of Mathematical and Statistical Psychology, 38(2), 197-201.

Tatti, E., Goyer, C., Burton, D. L., Wertz, S., Zebarth, B. J., Chantigny, M., Filion, M. 2015. Tillage management and seasonal effects on denitrifier community abundance, gene expression and structure over winter. Microbial Ecology, 70(3), 795-808.

Team, R. (2015). R-Studio: integrated development for R. R-Studio, Inc., Boston, MA, USA. In.

ter Horst, A. M., Santos-Medellín, C., Sorensen, J. W., Zinke, L. A., Wilson, R. M., Johnston, E. R., Trubl, G. G., Pett-Ridge, J., Blazewicz, S. J., Hanson, P. J. 2020. Minnesota peat viromes reveal terrestrial and aquatic niche partitioning for local and global viral populations. bioRxiv.

Thamdrup, B. 2012. New pathways and processes in the global nitrogen cycle. Annual Review of Ecology, Evolution, and Systematics, 43, 407-428.

Thingstad, T. F., Lignell, R. 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquatic microbial ecology, 13(1), 19-27.

Thoden, J. B., Holden, H. M. 2007. The molecular architecture of glucose-1-phosphate uridylyltransferase. Protein science, 16(3), 432-440.

Thompson, L. R., Zeng, Q., Kelly, L., Huang, K. H., Singer, A. U., Stubbe, J., Chisholm, S. W. 2011. Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. Proceedings of the National Academy of Sciences, 108(39), E757-E764.

Tim, U. S., Mostaghimi, S. 1991. Model for predicting virus movement through soils. Groundwater, 29(2), 251-259.

Timonen, S., Finlay, R. D., Olsson, S., Söderström, B. J. F. M. E. 1996. Dynamics of phosphorus translocation in intact ectomycorrhizal systems: non-destructive monitoring using a β-scanner. 19(3), 171-180.

Toda, M., Uchida, Y. 2017. Long-term use of green manure legume and chemical fertiliser affect soil bacterial community structures but not the rate of soil nitrate decrease when excess carbon and nitrogen are applied. Soil Research, 55(6), 524-533.

Torsvik, V., Øvreås, L. 2002. Microbial diversity and function in soil: from genes to ecosystems. Current opinion in microbiology, 5(3), 240-245.

Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urich, T., Engel, M., Schloter, M., Wagner, M., Richter, A. 2011. Nitrososphaera viennensis, an ammonia oxidizing archaeon from soil. Proceedings of the National Academy of Sciences, 108(20), 8420-8425.

Treseder, K. 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. 11(10), 1111-1120.

Trevors, J. J. W., Air,, Pollution, S. 1998. Bacterial biodiversity in soil with an emphasis on chemically-contaminated soils. 101(1-4), 45-67.

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H. W., Anderson, I. C., Jeffries, T. C., Zhou, J. Z., Singh, B. K. 2016. Microbial regulation of the soil carbon cycle: evidence from gene-enzyme relationships. Isme Journal, 10(11), 2593-2604.

Trubl, G., Hyman, P., Roux, S., Abedon, S. T. 2020. Coming-of-age characterization of soil viruses: A user's guide to virus isolation, detection within metagenomes, and viromics. Soil Systems, 4(2), 23.

Trubl, G., Jang, H. B., Roux, S., Emerson, J. B., Solonenko, N., Vik, D. R., Solden, L., Ellenbogen, J., Runyon, A. T., Bolduc, B. 2018a. Soil viruses are underexplored players in ecosystem carbon processing. Msystems, 3(5).

Trubl, G., Jang, H. B., Roux, S., Emerson, J. B., Solonenko, N., Vik, D. R., Solden, L., Ellenbogen, J., Runyon, A. T., Bolduc, B., Woodcroft, B. J., Saleska, S. R., Tyson, G. W., Wrighton, K. C., Sullivan, M. B., Rich, V. I. 2018b. Soil Viruses Are Underexplored Players in Ecosystem Carbon Processing. Msystems, 3(5).

Trubl, G., Roux, S., Solonenko, N., Li, Y.-F., Bolduc, B., Rodríguez-Ramos, J., Eloe-Fadrosh, E. A., Rich, V. I., Sullivan, M. B. 2019. Towards optimized viral metagenomes for double-stranded and single-stranded DNA viruses from challenging soils. PeerJ, 7, e7265.

Urakami, T., Sasaki, J., Suzuki, K.-I., Komagata, K. 1995. Characterization and Description of Hyphomicrobium denitrificans sp. nov. International Journal of Systematic and Evolutionary Microbiology, 45(3), 528-532.

Uritskiy, G. V., DiRuggiero, J., Taylor, J. 2018. MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome, 6(1), 1-13.

Van Bruggen, A. H., Semenov, A. M. 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology, 15(1), 13-24.

Vanarsdall, A. L., Okano, K., Rohrmann, G. F. 2006. Characterization of the role of very late expression factor 1 in baculovirus capsid structure and DNA processing. Journal of virology, 80(4), 1724-1733.

Vasickova, P., Pavlik, I., Verani, M., Carducci, A. 2010. Issues concerning survival of viruses on surfaces. Food and Environmental Virology, 2(1), 24-34.

Vaughan, D., Malcolm, R. 2012. Soil organic matter and biological activity (Vol. 16): Springer Science & Business Media.

Verbaendert, I., Boon, N., De Vos, P., Heylen, K. 2011. Denitrification is a common feature among members of the genus Bacillus. Systematic and Applied Microbiology, 34(5), 385-391.

Vukicevich, E., Lowery, T., Bowen, P., Urbez-Torres, J. R., Hart, M. 2016a. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development, 36(3).

Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J. R., Hart, M. 2016b. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development, 36(3), 1-14.

Wan, W., Tan, J., Wang, Y., Qin, Y., He, H., Wu, H., Zuo, W., He, D. 2020. Responses of the rhizosphere bacterial community in acidic crop soil to pH: Changes in diversity, composition, interaction, and function. Science of the total environment, 700, 134418.

Wang, F., Chen, S., Wang, Y., Zhang, Y., Hu, C., Liu, B. 2018a. Long-term nitrogen fertilization elevates the activity and abundance of nitrifying and denitrifying microbial communities in an upland soil: implications for nitrogen loss from intensive agricultural systems. Frontiers in Microbiology, 9, 2424.

Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and environmental microbiology, 73(16), 5261-5267.

Wang, Q., Guan, Z., Pei, K., Wang, J., Liu, Z., Yin, P., Peng, D., Zou, T. 2018b. Structural basis of the arbitrium peptide–AimR communication system in the phage lysis–lysogeny decision. Nature Microbiology, 3(11), 1266-1273.

Wang, W., Yang, M., Shen, P., Zhang, R., Qin, X., Han, J., Li, Y., Wen, X., Liao, Y. 2019. Conservation tillage reduces nitrous oxide emissions by regulating functional genes for ammonia oxidation and denitrification in a winter wheat ecosystem. Soil and Tillage Research, 194, 104347.

Wang, Y., Li, C., Tu, C., Hoyt, G. D., DeForest, J. L., Hu, S. 2017. Long-term no-tillage and organic input management enhanced the diversity and stability of soil microbial community. Science of the total environment, 609, 341-347.

Wardle, D. A., Verhoef, H. A., Clarholm, M. J. G. C. B. 1998. Trophic relationships in the soil microfood-web: Predicting the responses to a changing global environment. 4(7), 713-727.

Warton, D. I., Wright, S. T., Wang, Y. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution, 3(1), 89-101.

Weil, R. R., Islam, K. R., Stine, M. A., Gruver, J. B., Samson-Liebig, S. E. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture, 3-17.

Weinbauer, M. G., Rassoulzadegan, F. 2004a. Are viruses driving microbial diversification and diversity? Environ Microbiol, 6(1), 1-11.

Weinbauer, M. G., Rassoulzadegan, F. J. E. m. 2004b. Are viruses driving microbial diversification and diversity? , 6(1), 1-11.

Wilhelm, S. W., Suttle, C. A. 1999. Viruses and nutrient cycles in the sea: viruses play critical roles in the structure and function of aquatic food webs. BioScience, 49(10), 781-788.

Williamson, K. E., Fuhrmann, J. J., Wommack, K. E., Radosevich, M. 2017a. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. Annual review of virology, 4, 201-219.

Williamson, K. E., Fuhrmann, J. J., Wommack, K. E., Radosevich, M. J. A. r. o. v. 2017b. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. 4, 201-219.

Williamson, K. E., Radosevich, M., Smith, D. W., Wommack, K. E. 2007. Incidence of lysogeny within temperate and extreme soil environments. Environmental microbiology, 9(10), 2563-2574.

Williamson, K. E., Radosevich, M., Wommack, K. E. 2005a. Abundance and diversity of viruses in six Delaware soils. Appl Environ Microbiol, 71(6), 3119-3125.

Williamson, K. E., Radosevich, M., Wommack, K. E. 2005b. Abundance and diversity of viruses in six Delaware soils. Applied and environmental microbiology, 71(6), 3119-3125.

Williamson, K. E., Schnitker, J. B., Radosevich, M., Smith, D. W., Wommack, K. E. 2008. Cultivation-based assessment of lysogeny among soil bacteria. Microbial Ecology, 56(3), 437-447.

Wilson, W. A., Roach, P. J., Montero, M., Baroja-Fernández, E., Muñoz, F. J., Eydallin, G., Viale, A. M., Pozueta-Romero, J. 2010. Regulation of glycogen metabolism in yeast and bacteria. FEMS microbiology reviews, 34(6), 952-985.

Winget, D. M., Wommack, K. E. 2008. Randomly amplified polymorphic DNA PCR as a tool for assessment of marine viral richness. Appl Environ Microbiol, 74(9), 2612-2618.

Wommack, K. E., Colwell, R. R. J. M., reviews, m. b. 2000. Virioplankton: viruses in aquatic ecosystems. 64(1), 69-114.

Wommack, K. E., Nasko, D. J., Chopyk, J., Sakowski, E. G. 2015. Counts and sequences, observations that continue to change our understanding of viruses in nature. Journal of Microbiology, 53(3), 181-192.

Wortman, S. E., Drijber, R. A., Francis, C. A., Lindquist, J. L. 2013. Arable weeds, cover crops, and tillage drive soil microbial community composition in organic cropping systems. Applied Soil Ecology, 72, 232-241.

Wu, Y., Lu, L., Wang, B., Lin, X., Zhu, J., Cai, Z., Yan, X., Jia, Z. 2011. Long-term field fertilization significantly alters community structure of ammonia-oxidizing bacteria rather than archaea in a paddy soil. Soil Science Society of America Journal, 75(4), 1431-1439.

Wu, Y.-W., Simmons, B. A., Singer, S. W. 2016. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. Bioinformatics, 32(4), 605-607.

Wu, Y.-W., Tang, Y.-H., Tringe, S. G., Simmons, B. A., Singer, S. W. 2014. MaxBin: an automated binning method to recover individual genomes from metagenomes using an expectation-maximization algorithm. Microbiome, 2(1), 1-18.

Xia, X., Zhang, P., He, L., Gao, X., Li, W., Zhou, Y., Li, Z., Li, H., Yang, L. 2019. Effects of tillage managements and maize straw returning on soil microbiome using 16S rDNA sequencing. Journal of integrative plant biology, 61(6), 765-777.

Xiang, S.-R., Doyle, A., Holden, P. A., Schimel, J. P. J. S. B., Biochemistry. 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biology and Biochemistry, 40(9), 2281-2289.

Xue, K., Xie, J., Zhou, A., Liu, F., Li, D., Wu, L., Deng, Y., He, Z., Van Nostrand, J. D., Luo, Y. 2016. Warming alters expressions of microbial functional genes important to ecosystem functioning. Frontiers in Microbiology, 7, 668.

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., Zhang, Y. 2015. The I-TASSER Suite: protein structure and function prediction. Nature Methods, 12(1), 7-8.

Yeager, J. G., O'Brien, R. T. 1979a. Enterovirus inactivation in soil. Appl Environ Microbiol, 38(4), 694-701.

Yeager, J. G., O'Brien, R. T. 1979b. Structural changes associated with poliovirus inactivation in soil. Appl Environ Microbiol, 38(4), 702-709.

Yoo, S.-H., Lee, B.-H., Moon, Y., Spalding, M. H., Jane, J.-l. 2014. Glycogen synthase isoforms in Synechocystis sp. PCC6803: identification of different roles to produce glycogen by targeted mutagenesis. PLoS One, 9(3), e91524.

Yuan, Z., Chen, H. Y. 2012. A global analysis of fine root production as affected by soil nitrogen and phosphorus. Proceedings of the Royal Society B: Biological Sciences, 279(1743), 3796-3802.

Zabaloy, M. C., Allegrini, M., Tebbe, D. A., Schuster, K., Gomez, E. d. V. 2017. Nitrifying bacteria and archaea withstanding glyphosate in fertilized soil microcosms. Applied Soil Ecology, 117, 88-95.

Zablocki, O., Adriaenssens, E. M., Cowan, D. 2016. Diversity and Ecology of Viruses in Hyperarid Desert Soils. Appl Environ Microbiol, 82(3), 770-777.

Zablocki, O., Jang, H. B., Bolduc, B., Sullivan, M. B. (2019). *vConTACT 2: A Tool to Automate Genome-Based Prokaryotic Viral Taxonomy.* Paper presented at the Plant and Animal Genome XXVII Conference (January 12-16, 2019).

Zablocki, O., van Zyl, L., Adriaenssens, E. M., Rubagotti, E., Tuffin, M., Cary, S. C., Cowan, D. 2014. High-level diversity of tailed phages, eukaryote-associated viruses, and virophage-like elements in the metaviromes of antarctic soils. Appl Environ Microbiol*,* 80(22), 6888-6897.

Zahran, H. H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and molecular biology reviews*,* 63(4), 968-989.

Zak, J. C., Willig, M. R., Moorhead, D. L., Wildman, H. G. J. S. B., Biochemistry. 1994. Functional diversity of microbial communities: a quantitative approach. 26(9), 1101-1108.

Zhalnina, K., Dias, R., de Quadros, P. D., Davis-Richardson, A., Camargo, F. A., Clark, I. M., McGrath, S. P., Hirsch, P. R., Triplett, E. W. 2015a. Soil pH determines microbial diversity and composition in the park grass experiment. Microbial Ecology*,* 69(2), 395-406.

Zhalnina, K., Dias, R., de Quadros, P. D., Davis-Richardson, A., Camargo, F. A., Clark, I. M., McGrath, S. P., Hirsch, P. R., Triplett, E. W. J. M. e. 2015b. Soil pH determines microbial diversity and composition in the park grass experiment. 69(2), 395-406.

Zhang, C., Song, Z., Zhuang, D., Wang, J., Xie, S., Liu, G. 2019a. Urea fertilization decreases soil bacterial diversity, but improves microbial biomass, respiration, and N-cycling potential in a semiarid grassland. Biology and Fertility of Soils*,* 55(3), 229-242.

Zhang, H., Zhao, Z., Li, S., Chen, S., Huang, T., Li, N., Yang, S., Wang, Y., Kou, L., Zhang, X. 2019b. Nitrogen removal by mix-cultured aerobic denitrifying bacteria isolated by ultrasound: Performance, co-occurrence pattern and wastewater treatment. Chemical Engineering Journal, 372, 26-36.

Zhang, L., Jiang, M., Ding, K., Zhou, S. 2019c. Iron oxides affect denitrifying bacterial communities with the nirS and nirK genes and potential N2O emission rates from paddy soil. European Journal of Soil Biology, 93, 103093.

Zhou, J., Jiang, X., Wei, D., Zhao, B., Ma, M., Chen, S., Cao, F., Shen, D., Guan, D., Li, J. 2017. Consistent effects of nitrogen fertilization on soil bacterial communities in black soils for two crop seasons in China. Sci Rep, 7(1), 3267.

Zhou, L., Wang, S., Zou, Y., Xia, C., Zhu, G. 2015. Species, abundance and function of ammonia-oxidizing archaea in inland waters across China. Scientific reports, 5(1), 1-13.

Zhuang, J., Jin, Y. 2003a. Virus retention and transport as influenced by different forms of soil organic matter. Journal of environmental quality, 32(3), 816-823.

Zhuang, J., Jin, Y. 2003b. Virus retention and transport through Al-oxide coated sand columns: effects of ionic strength and composition. Journal of Contaminant Hydrology, 60(3-4), 193-209.

Zucker, M., Hankin, L. 1970. Regulation of pectate lyase synthesis in Pseudomonas fluorescens and Erwinia carotovora. Journal of bacteriology, 104(1), 13-18.

Appendix 4

Table 4.1 Sequencing information of each soil sample.

Sample	Sample ID	Total PE reads	Total assembled contigs (>1kbp)	Total assembled reads	Assembled percentage	Total viral contigs	Total virus reads
NCNTN0_1	M35	78,713,410	223,037	2,599,218	1.65%	6,881	77,034
NCNTN0_2	M63	58,385,517	131,301	1,339,845	1.15%	3,539	34,772
NCNTN0_4	M107	61,062,926	130,169	1,422,914	1.17%	4,606	49,419
NCNTN60_1	M56	67,481,448	186,806	2,109,378	1.56%	4,621	49,061
NCNTN60_2	M9	81,710,978	195,748	2,328,660	1.42%	5,415	60,388
NCNTN60_4	M77	67,072,458	197,580	2,161,159	1.61%	4,534	47,917
VNTN0_1	M37	76,463,359	214,116	2,584,629	1.69%	5,930	66,678
VNTN0_2	M62	67,539,582	180,686	2,028,163	1.50%	5,423	57,828
VNTN0_4	M112	51,805,430	109,048	1,204,616	1.16%	2,592	27,152
VNTN60_1	M51	90,119,576	284,218	3,441,075	1.91%	7,305	86,666
VNTN60_2	M16	61,552,488	140,778	1,645,990	1.34%	3,675	40,775
VNTN60_4	M79	73,928,033	167,628	1,882,959	1.27%	4,539	48,176

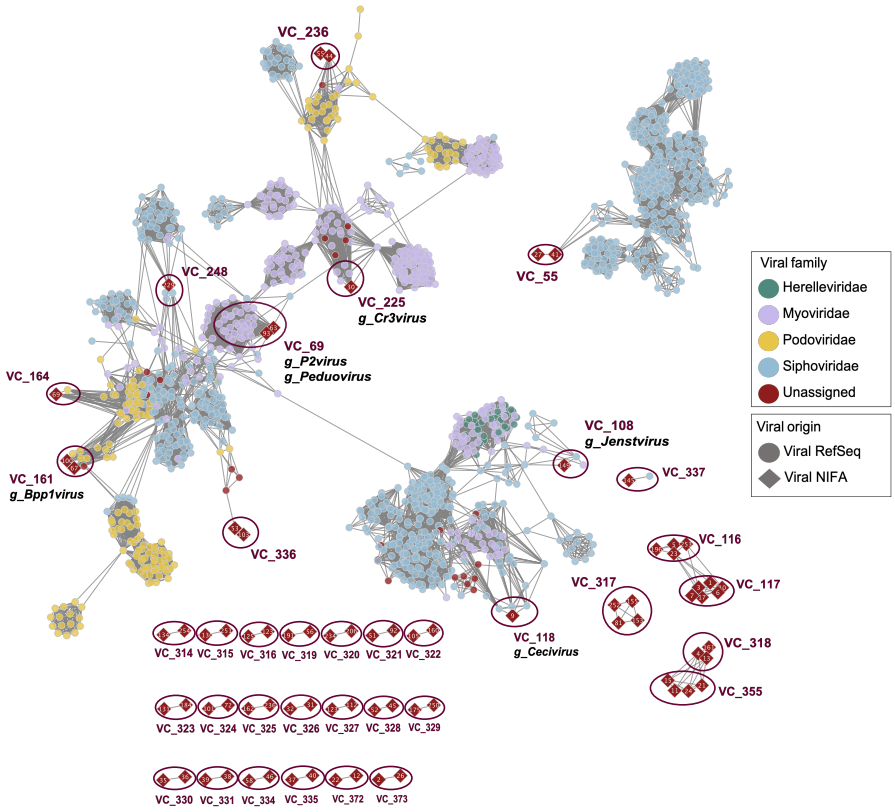


Figure 4.1 Gene-sharing network of vOTUs.

Recovered vOTUs clustered with RefSeq (v 94) viral genomes or fragments. The diamond shape with red colors represents the vOTUs recovered in our studies. Each node with round shape is the viruses obtained from RefSeq database. Nodes with round shape with green, purple, yellow, blue colors indicated the major viral families in the database. Viral genome pairs with sufficiently similar (significance score greater than 1) were joined by an edge in grey. The vOTUs classified as "outliers" were not shown in the network.

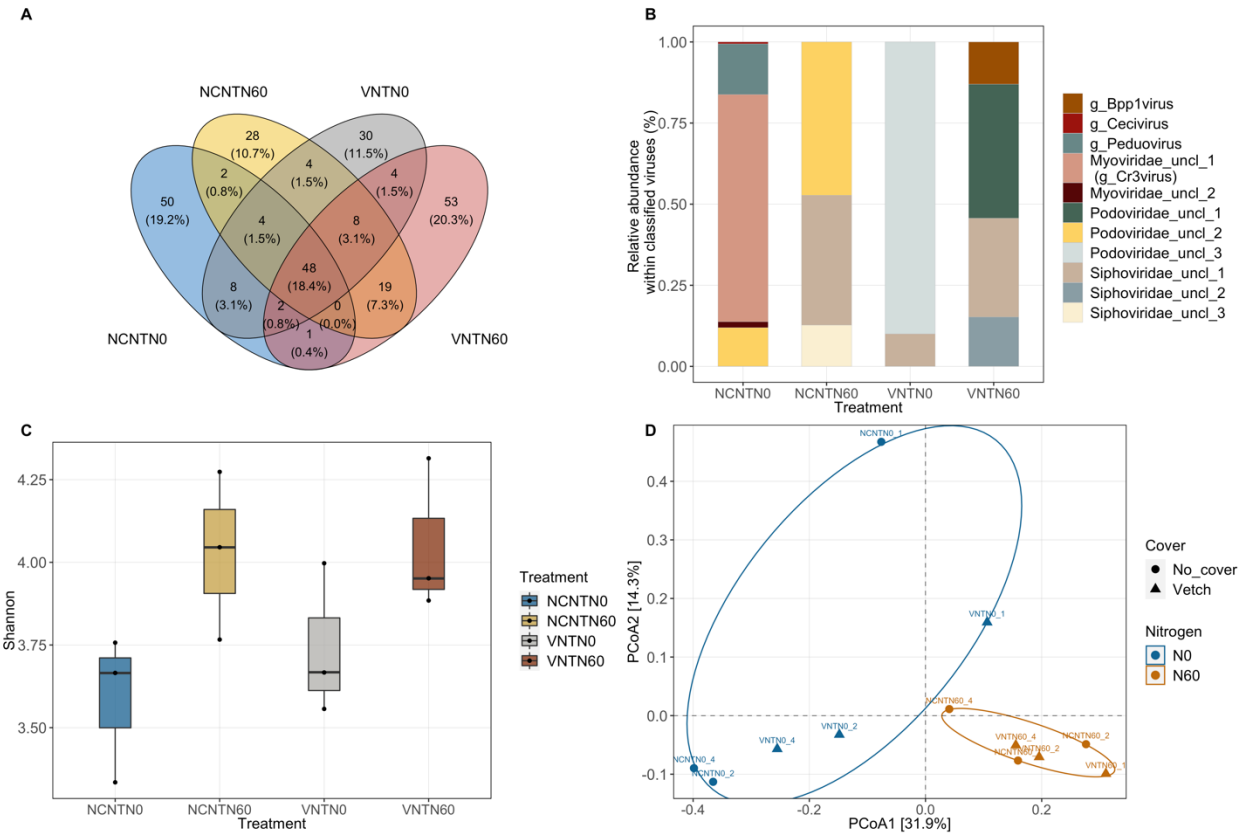


Figure 4.2 The distribution and diversity of vOTUs recovered in this study across four treatments.

Figure 2A Distribution of vOTUs across NCNTN0, NCNTN60, VNTN0, VNTN60 treatments based on read mapping to the updated PIGEON database. Figure 2B Stacked bar plot indicating the relative abundance of viral taxa in each treatment, only considering 11 viral taxa identified using the gene-sharing network (e.g., Podovirdae_uncl_1 and Podovirdae_uncl_2 have same family but different genus). Figure 2C Shannon index for 260 vOTUs across 4 treaments. Figure 2D Principal coordinates analysis (PCoA) of viral community composition derived from read mapping to viral contigs with Bray-Cutis dissimilarities. A permutational multivariate analysis of variance (PERMANOVA) with 2000 permutations was applied. The abundance of total 260 vOTUs difference was significant when grouping by fertilization management (No interaction

effect). NCNTN0 represent no-cover, no-tilled and no fertilization; VNTN60_4 is vetch-covered, no-tilled with fertilization.

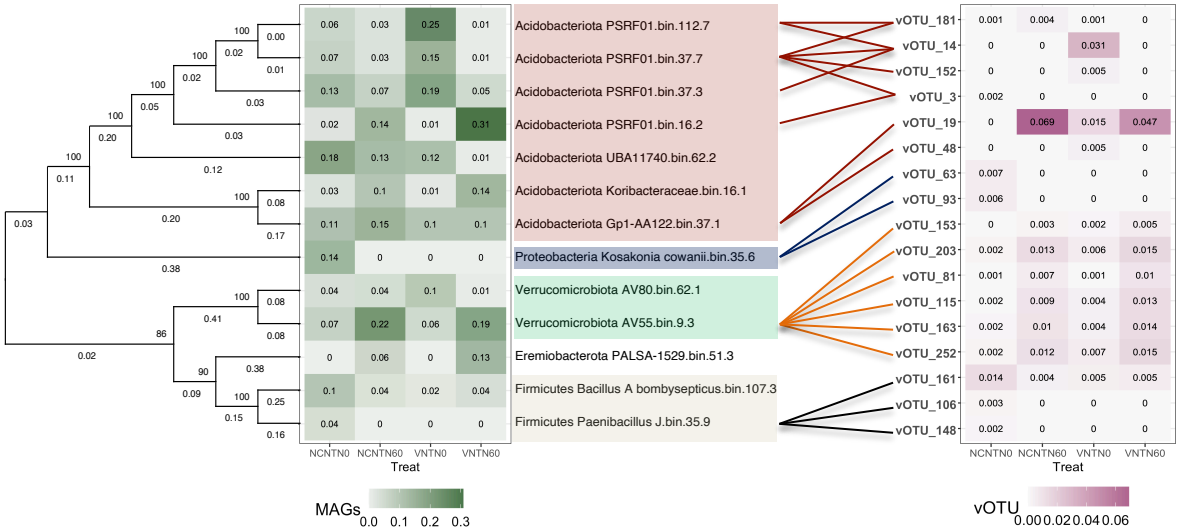


Figure 4.3 The link between virus and bacterial host based on the results using CRISPR and BLAST.

Unrooted phylogenetic tree of metagenome-assembled genomes (MAGs) under bacterial domain. The tree was built from concatenated protein sequences generated by alignment with single-copy gene defined by CheckM. Bootstrap values were shown as integer number near to each node, representing phylogenetic confidence of the tree topology. Branch length shown as decimal indicate the genetic change. The heatmaps represent relative abundance of bacterial genome (blue) and vOTUs (red) in each treatment. The bacterial host and virus were connected according to the CIRSPR and BLAST analysis (Table S4 and S5). The background color on taxonomic names indicated the same bacterial phyla.

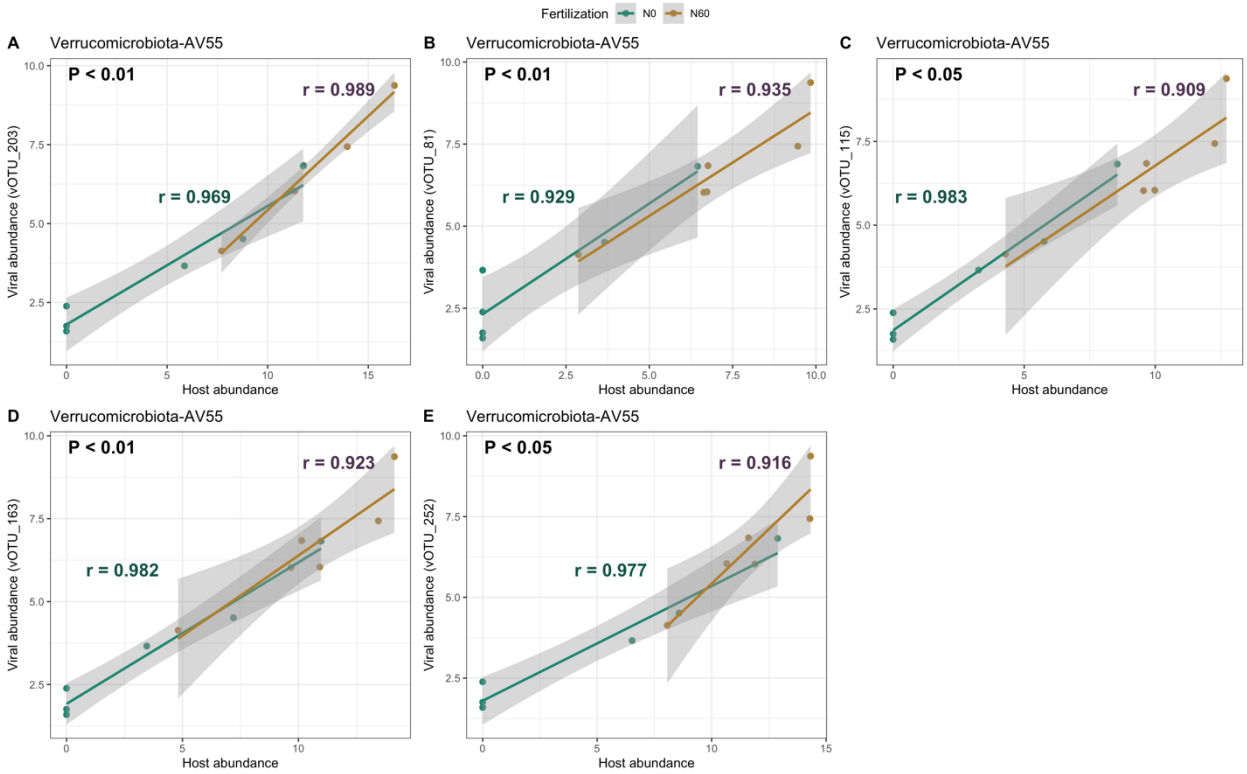


Figure 4.4 Correlation between abundance of five vOTUs and their host *Verrucomicrobiota AV55*.

Green and yellow color represent no fertilization and fertilization, respectively. The viral and host abundance is calculated as normalized mean coverage depth. Correlation coefficient (r) were calculated by Pearson's product-moment correlation. The normality of data was checked by Shapiro - Wilk Normality Test before the correlation analysis. The grey area showed 95% confidence interval.

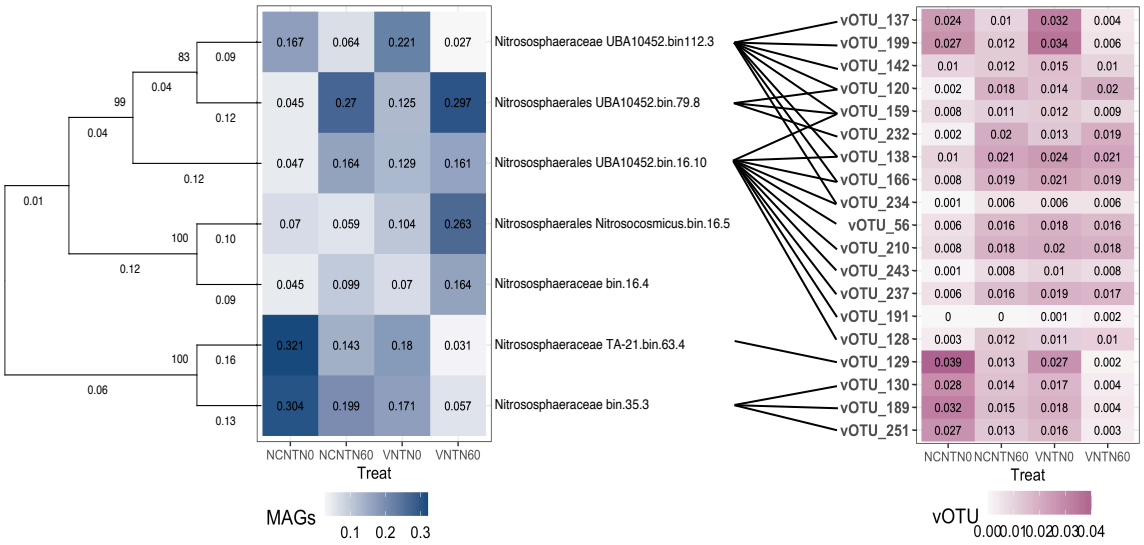


Figure 4.5 The link between viruses and archaeal host based on the results using BLAST.

Archaeal genomes affiliated with *Thermoproteota*. Unrooted phylogenetic tree of metagenome-assembled genomes (MAGs) under bacterial domain. The tree was built from concatenated protein sequences generated by alignment with single-copy gene defined by CheckM. Bootstrap values were shown as integer number near to each node, representing phylogenetic confidence of the tree topology. Branch length shown as decimal indicate the genetic change. The heatmaps represent average relative abundance of every archaeal genome or vOTUs in every treatment.

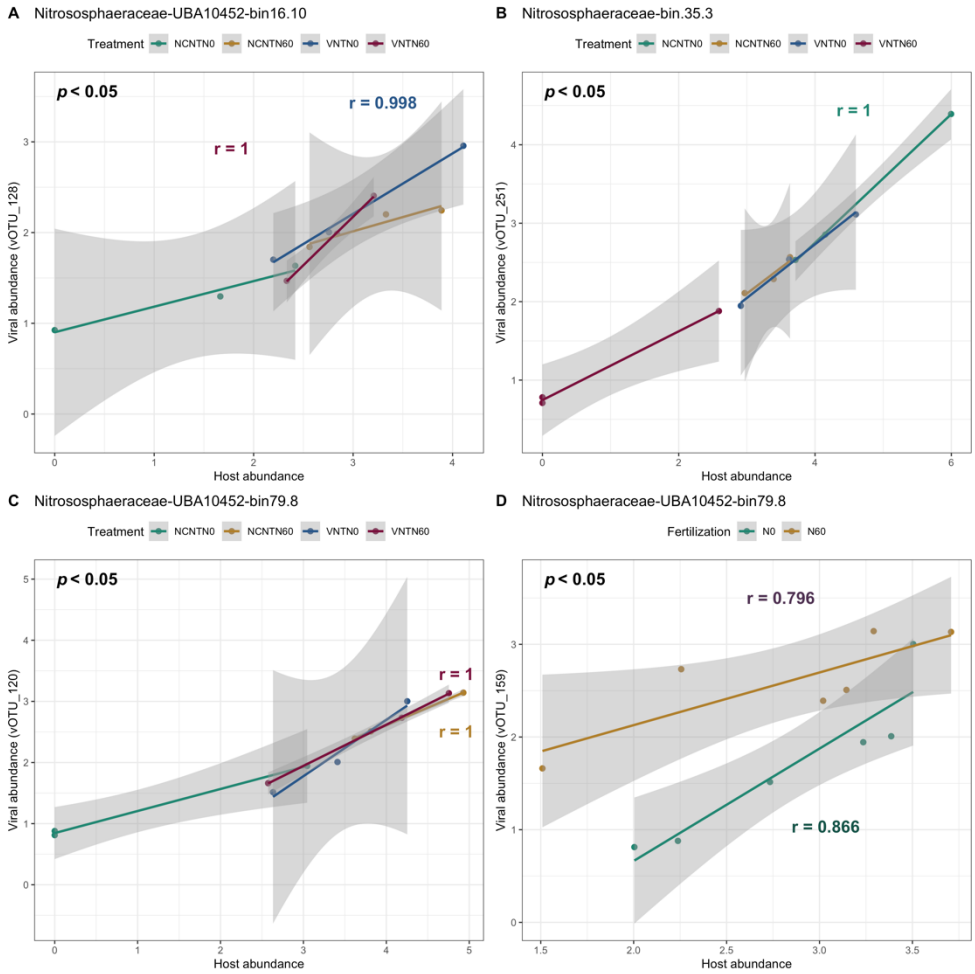


Figure 4.6 Correlations between vOTUs and their archaeal host *Thermoproteota Nitrososphaeraceae*.

Green, yellow, blue, and red in figure 6A to 6C represent NCNTN0, NCNTN60, VNTN0, and VNTN60, respectively. Green and yellow color in figure 6D represent no fertilization and fertilization, respectively. The viral and host abundance is calculated as normalized mean coverage depth. A square-root transformation was used before correlation analysis. Correlation coefficient (r) were calculated by Pearson's product-moment correlation. The normality of data was checked by Shapiro - Wilk Normality Test before the correlation analysis.

Chapter 5 The influence of long-term cover cropping and inorganic N fertilization on microbial carbon, nitrogen cycling genes and viral auxiliary metabolic genes

A version of this chapter is in preparation for submission to Applied Soil Ecology

by Ning Duan [a], Mark Radosevich [a], Jie Zhuang [a, c], Jennifer M. DeBruyn [a], Margaret E. Staton [b], Sean M. Schaeffer [a,*]

Contribution statement

Ning Duan carried out the experiment, molecular experiments, and all data processing and statistical analysis. Jie Zhuang, Mark Radosevich, Jennifer M. DeBruyn, Margaret E. Staton, Sean M. Schaeffer are responsible for the project and help with the revision of the manuscript.

[a] Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

[b] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, United States

[c] Center for Environmental Biotechnology, The University of Tennessee, Knoxville, TN 37996, United States

* Corresponding author. Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN, 37996, United States

Abstract

Agricultural soils are an important repository of carbon (C) and nitrogen (N), and microbes plays central roles in C and N fate. Virus modulation of microbial metabolism has been increasingly recognized. However, technical limitations on soil viral research restrict the knowledge of the

link between virus and microbes, and the influence of viruses in C cycle processes. Here, we identify genes encoding enzymes used to decompose chemically labile to recalcitrant C component (starch, hemicellulose, cellulose, chitin, pectin, and lignin) degradation and N-cycling genes from bulk soil microbial communities, and Auxiliary Metabolic Genes (AMGs) from soil viral populations using metagenomics. Soil samples were collected from an over 40-year continuous cotton plantation system under two management practices including cover cropping (hairy vetch, *Vicia villosa* Roth) and fertilization (ammonia nitrate, 67 kg/ha). Gene relative abundances for decomposing cellulose ($p < 0.001$) and hemicellulose ($p < 0.001$) increased in fertilized plots compared to no fertilized plots. The abundances of lignin degrading genes increased when no N fertilizer was applied compared to fertilized soil ($p < 0.01$). Pectin ($p < 0.05$) and chitin ($p < 0.05$) decomposition genes were more abundant when no cover crops were used. Genes involved in nitrification, denitrification, and dissimilatory nitrate reduction increased in abundance in fertilized soil compared to controls. Strong positive correlations among gene relative abundances nitrification, denitrification, and dissimilatory nitrate reduction processes were observed in N fertilizer applied soil, which may be important for balancing of N loss and N retention. In terms of viruses influencing C metabolism, the AMGs glycogen synthase. Viral *gmd*, *glf* were orginated from *Cyanobacteria*, *Actinobacteria*, and *Planctomycetes*, respectively, and viral *galE* was related to *Actinobacteria* and *Proteobacteria*. These four viral AMGs with ability of mediate host C metabolism, which may involve in energy production and storage, lipopolysaccharides (LPS), and extracellular polymeric substances (EPS) formation, improve regular progress of infection or fitness (host receptor recognition, penetration), and the ability to manipulate the host metabolism to build defense mechanisms (host biofilm formation). Together, our findings highlight the importance of microbial on N

fertilization and cover cropping on maintaining long-term C pool stability and N content in agricultural soil, and the roles of viruses on C metabolism through regulating microbial metabolism using auxiliary metabolic genes.

5.1 Introduction

Soils are important carbon reservoirs and nutrient resources containing approximately 1462 to 1548 Pg of C and 133 to 140 Pg of N in the upper 100 cm of layers (Batjes, 2014; Paustian et al., 2019). Carbon sequestration and nitrogen retention are important on keeping soil fertility and productivity (Paustian et al., 2019; Sun & Badgley, 2019).

Agricultural managements contribute to the dynamic balance of C and N cycling by controlling the composition of organic matter, soil physical proprieties, and shifting of microbial functional groups (Martínez-García et al., 2018). Fertilizers promote crop above and below ground growth and increased total soil N and organic C input (Salinas-Garcia et al., 1997; Raun et al., 1998; LeBauer & Treseder, 2008; Yuan & Chen, 2012). Cover crops not only add freshly plant residues, which is important on native organic matter protection and positive effect on organic matter building up (Smith, 1966; Cheshire, 1985), but influence the C:N ratio of organic matter to drive the dynamics of mineralization and immobilization, and potentially shifting the soil microbial communities (Acosta-Martínez et al., 2010; Wortman et al., 2013).

Soil microorganisms contribute to regulating global C decomposition and N cycling due to its functional diversity and capability adaptation on environmental change (Liang et al., 2017a; Che et al., 2018). This functional variation can be observed by estimating abundances of functional gene markers (Ouyang et al., 2018). Carbohydrates in the form of polysaccharides, are one of the

largest fractions of organic matter in soil and play a vital role in biological nutrition for microorganisms (Bacon, 1968; Cheshire, 1985). N as an essential nutrient element affecting the C degradation and sequestration (LeBauer & Treseder, 2008; Homyak et al., 2017; Averill & Waring, 2018). Therefore, it is critical consider N-cycling genes in order to predict the N input and losses. The equilibrium between N deposition and N loss influences N availability for soil microbes and plant uptake. Nitrification and denitrification result the N loss as the form of nitrate through leaching or N gases released from agricultural soils (Philippot et al., 2007; Norton & Stark, 2011; Ouyang et al., 2018). Nitrogen reduction as N resource serve the growth of microbes (assimilatory nitrate reduction) or as electron acceptor for anaerobic respiration in microbes (dissimilatory nitrate reduction) for energy conservation (Kraft et al., 2011; Thamdrup, 2012). Increased inorganic N may impede the oxidative enzyme production in microbes leading to the decreasing on recalcitrant SOM (Moorhead & Sinsabaugh, 2006; Prescott, 2010; Jian et al., 2016; Averill & Waring, 2018).

Increasing evidence shows that the potential for "top-down" control by soil viruses could occur in soils which may lead to shifts in microbial function and affect the nutrient and energy flows in soils (Williamson et al., 2017a). Indeed, the roles of viruses involved in N and C-cycling on microbial host in soil has been received increased attention (Emerson et al., 2018; Trubl et al., 2018a; Starr et al., 2019; ter Horst et al., 2020). In soils, more than 10^{10} viral entities can be harbored in one gram of soil (Ashelford et al., 2003; Williamson et al., 2017a). In addition to driving host mortality, viruses can reprogram vital microbial metabolism through auxiliary metabolic genes (AMGs) (Thompson et al., 2011; Enav et al., 2014; Huang et al., 2021). AMGs on bacteriophages were originated from direct infected host or distantly related bacteria (Huang

et al., 2021). In marine system, cyanophages are carrying AMGs with diverse functions, including photosynthesis, carbon metabolism, nucleic acid metabolism, stress tolerance, etc. The viral AMGs can be obtained from *Synechococcus* and *Prochlorococcus* (Crummett et al., 2016). In soil system, most of the AMG genes identified in soil participate in carbohydrate, amino acid, and energy metabolism (Emerson et al., 2018; Trubl et al., 2018a; Graham et al., 2019; ter Horst et al., 2020). The importance of viral community on the

Currently, the complexity of soil environment, unevenly distribution of soil microbes and viruses result in a great challenge to study the soil viral community and their microbial host. Therefore, functions of soil viruses, the potential effect on microbial hosts, and the association between two communities in soils are still limited. In this study, we incorporate viral AMGs and microbial C metabolism and N-cycling genes to explore the contribution of soil viruses and microbes on functional changes in C and N cycles in an agroecosystem. We expected that greater abundance of recalcitrant C degradation gene would be observed under no fertilization treatment because the microbes tend to obtain N by mining recalcitrant organic matter the under N limitation status. Further, we expected that vetch residues would increase labile organic matter input and increase the gene abundances for decomposing labile organic C for microbial metabolism. Also, N transformation including nitrification, denitrification, and nitrate reduction would response to N fertilizer application. AMGs for carbohydrate metabolism could be found and linked to the microbial host. In this study, we used metagenomics to reveal potential viral function from the aspects of Auxiliary metabolic genes based on the viral OTUs identified in the previous chapter.

5.2 Material and methods

5.2.1 Site description and sample collection

The work was conducted by sampling an on-going long-term conservation management experimental site established in 1981 in West Tennessee. The plot study is a randomized complete block (RCBD) with a split plot design. Inorganic nitrogen fertilizer (ammonium nitrate, NH_4NO_3) was applied at two nitrogen levels (0 and 67 kg/ha) as the main plots and were divided into sub-plots that contained two levels of cover crop treatments (hairy vetch, *Vicia villosa* Roth, and no cover), no-tillage will be considered in this study. All 4 treatments with 3 replications were sampled on May, 21st, 2019, shortly after burn-down of the cover crop and just before planting in spring. The samples were coded with the following form: “type of cover crop”, “no-tillage”, “fertilization or not” and “serial number of replicates”, e.g. NCNTN0_1 represent no-cover, no-till, and no fertilization treatment of replicates #1; VNTN60_4 is vetch-covered, no-till with fertilization, replicates #4. Bulk soil metagenomic sequencing was applied on 12 samples (see details in Duan et al 2021 and below).

5.2.2 DNA extraction, library construction, and metagenomic sequencing

Soil samples were collected from the depth range 0 to 10cm using a 2.5cm diameter soil probe. The samples were stored in the lab freezer (-20 ℃) for DNA extraction the next day. Total soil DNA was extracted using DNeasy® PowerLyzer® Powersoil® kit (REF 12855, QIAGEN) from 0.25 g soil per sample following the manufacturer’s instructions. The DNA library were built in

Institute for Genome Science, School of Medicine in University of Maryland. Briefly, Genomic DNA libraries were constructed for sequencing on the Illumina platform using KAPA Library Preparation Kit (Kapa Biosystems, Woburn, MA). The libraries were pooled and sequenced on Illumina NovaSeq6000 S2 using 150bp pair-end reads (0.5 S2-lane, 900M read pairs, 275 Gbp yield average, Illumina, San Diego, CA) at the institute for genome sciences, school of Medicine at the University of Maryland. The pipeline triggers trimming when either 1% of reads have >=3 consecutive bases < Q20 or when adaptors are detected in >1% of the reads. More details see the previous chapter.

5.2.3 Reads pre-processing and assembly, and functional gene annotation

Cutadapt (version 1.18) was used to remove the adapters (smallRNA, Illumine, Nextera), the Quality Phred score cutoff was 20, Maximum trimming error rate was 0.1, the reads shorter than 50bp were discarded (Martin, 2011). *De novo* assembly with MEGAHIT (Li et al., 2015; Li et al., 2016). QUAST(v.5.0.2) was used to estimate contigs statistics (Mikheenko et al., 2018).

The protein-coding genes were predicted and annotated using Distilled and Refined Annotation of Metabolism (DRAM) pipeline. Briefly, assembly derived FASTA files were as input file and open reading frames (ORFs) were detected by Prodigal, amino acid sequences were searched and predicted against the KOfam using MMseqs2. And HMM profile searches of the Pfam database via MMSeqs2, and searches of dbCAN and VOGDB databases were performed by HHMER3, the hits with coverage length greater than 35% of the model and e-value < 10^{-15} were retained. Functional annotation of genes was annotated against Kyoto Encyclopedia of Genes and

Genomes (KEGG) database and Pfam database. After the gene annotation, DRAM strainer function was used to pull the genes under CAZy, hydrocarbon decomposition, nitrogen cycling categories. The genes shorter than 100 bp were discarded. The genes from all the samples were collected in each category. And the gene in each category were clustered using CD-HIT with global identity threshold of 0.95, alignment coverage for shorter contigs length more than 95% to remove the redundant sequences (dereplication) (Li et al., 2002; Li & Godzik, 2006; Evans & Denef, 2020). Paired end reads of each sample were mapped to each gene catalog using bbmap. The SAM file with alignment information of each sample was transformed to the bam format and bam files were sorted and indexed by SAMtools (Li et al., 2009) and BEDtools (Quinlan & Hall, 2010) to parse the bam file and for downstream generation of counts table by BamM. Customized R script were created for downstream analysis.

5.2.4 Identification of viral auxiliary metabolic genes (AMG) and phylogenetic analyses

Putative AMGs were detected by VIBRANT in vOTU sequences. All viral contigs generated by VirSorter, DeepVirfinder, and VIBRANT itself were analyzed by VIBRANT with default settings. All the protein sequences were alignment using MUSCLE (Edgar, 2004), and then we use maximum likelihood (ML)-based FastTree with 1000 bootstraps (Price et al., 2009). The GDP-mannose 4,6-dehydratase [EC:4.2.1.47], Glycogen synthase [EC:2.4.1.11], UDP-glucose 4-epimerase [EC:5.1.3.2], UDP-galactopyranose mutase [EC:5.4.99.9] genes can be identified from both non-viral contigs and vOTUs. Nearest neighbor (non-viral genes) of each AMGs based on phylogeny trees were selected. And then selected non-viral genes, AMGs and top 10

results searched from NCBI by BLASTp were used to constructed gene phylogenetic trees. The protein structure of each AMGs were predicted using I-TASSER (Yang et al., 2015).

5.2.5 Statistical analysis

Statistical analyses were conducted in R. To test the gene response of different process nitrogen cycling to fertilization and cover cropping managements, The Shapiro - Wilk Normality Test was performed to check the normality of relative abundances of each gene category (Shapiro et al., 1968). A mixed effect model was built using *lme4* R package, the block was random effect, fertilization and cover crops are regard as fixed effect. Type II Wald Chi-square tests were applied on the mixed model to test an alternative hypothesis. Tukey's HSD (Honest Significant Difference) was used for the post-hoc test (Abdi & Williams, 2010). A negative binomial distribution model in DESeq2 package were employed for detection of differentially expressed genes across the fertilization and cover crops with false discovery rate less than 0.1 (Love et al., 2014). Benjamini–Hochberg method were employed to adjust p-values for multi-comparison test (Benjamini & Hochberg, 1995). The regularized logarithm (rlog) transformation on count data were used prior for redundancy analysis (RDA) analysis with Euclidean distance.

5.3 Results

5.3.1 Effects of long-term fertilization and cover cropping on variation on abundances of functional genes related to carbon degradation

A total of 27 functional genes encoding enzymes for degrading starch, hemicellulose, cellulose, chitin, pectin, and lignin were examined across four treatments. Enzymes for decomposing different carbon substrate exhibited variation among four fertilization and cover cropping treatments (Fig. 5.1). Greater abundances of genes for cellulose ($p < 0.001$) and hemicellulose ($p < 0.001$) decomposition were found in fertilized compared to control plots (Fig. 5.1A). Conversely, genes for degrading lignin, which is chemically more recalcitrant, were significantly greater in no N fertilizer applied soil compared to fertilizer applied soil ($p < 0.01$). To examine cover cropping effects, pectin ($p < 0.05$) and chitin ($p < 0.05$) decomposition genes were more abundant in no crop covered than vetch covered soil (Fig. 5.1B).

We compared the fertilization with no cover cropping effect on C-degradation genes using differential abundances analysis. The 5,5'-dehydrodivanillate O-demethylase oxygenase subunit (*ligXa*) was significantly more abundant in control than fertilizer applied soil. Glucoamylase encoding enzymes for decomposing starch and endo-1,4-beta-xylanase for hemicellulose decomposition increased under fertilization. And genes coding for enzymes for starch degradation decreased with fertilization compared to the control treatment (Fig. 5.2A).

In soil with no fertilizer added, genes related to degradation of relatively recalcitrant carbon significantly increased. These included pectic acid lyase, related to decomposition of pectin, and the 5,5'-dehydrodivanillate O-demethylase oxygenase subunit involved in the catabolism of 5,5'-dehydrodivanillate (DDVA), which is an intermediate in the biodegradation of lignin component of lignin decomposition enzyme. We also observed that genes for beta-N-acetylglucosaminidase, a key enzyme for chitin decomposition, was also abundant in the no fertilization treatment. These results suggest that restriction of exogenous inorganic N input might stimulate genes for decomposing recalcitrant C component, and more gene abundances varied with fertilization when cover cropping exist (Fig. 5.2 and Table S5.2).

The response of gene abundances to cover cropping management showed that fewer genes varied between cover cropping and controls. More genes responded to N fertilizer addition whether or not the soil had vetch cover crops present (Fig. 5.2B). Genes for starch (alpha-glucosidase) increased when no fertilizer applied, and gene for hemicellulose (beta-glucuronidases) were abundant in no fertilization management (Fig. 5.2C). Two genes for decomposing lignin and chitin increased in no-cover than vetch-covered soil, which might associate with C limitation. Our results indicate that fertilization had a greater effect on influencing gene abundances of carbon decomposition than cover cropping treatment, and no cover cropping may increase more recalcitrant C genes abundances and may result in recalcitrant C decomposition.

Moreover, the variation of structure of genes for carbon decomposition across samples was revealed by redundancy analysis (RDA) (Fig. 5.3). The PERMNOVA (number of permutations= 9999) showed that fertilization management was a significant indicator for predicting variation

of genes related to C substrate decomposition. Together, the fertilization and cover cropping, and their interaction explained 54.51 % variation of variation of carbon genes among samples (Table S5.1).

5.3.2 Effects of long-term fertilization and cover cropping on variation on functional genes involved in N-cycling

The same analysis was performed on 19 genes involved in nitrification, denitrification, assimilatory nitrate reduction, and dissimilatory nitrate reduction, and nitrate/nitrite transportation. Under no cover cropping treatments, abundances of genes related to nitrification (*amoC*), denitrification (*norB*, *narI*), and dissimilatory nitrate reduction (*nirB*, *narI*) increased with inorganic N application (Fig. 5.5A and Table S5.4). In soils with cover crops, more genes involve in denitrification and dissimilatory nitrate reduction responded to inorganic N fertilizer input. *napB* (denitrification, dissimilatory nitrate reduction), *nrfH* (dissimilatory nitrate reduction) were more abundant in fertilization than control. Conversely, *nasA* encoding assimilatory nitrate reductase catalytic subunit were increased in no-fertilizer applied soil (Fig. 5.5B and Table S5.4).

Cover cropping showed weak or no influence on abundances of nitrogen cycling genes since only *nirB* (dissimilatory nitrate reduction) varied between vetch covered and no-cover under no addition of inorganic N (Fig. 5.5C), and redundancy analysis (RDA) and PERMANOVA supported the results that more than 53.15% variation of nitrogen cycling gene across samples was explained by fertilization (Table S5.2). Overall, the results indicate that inorganic nitrogen

input more importantly explains variations of gene abundance related to nitrification, denitrification and nitrate reduction compared with a N-fixing cover crop.

Due to the weak effect from cover cropping on N cycling genes, we only examined correlations of genes within functional category in fertilization and control samples. Strong positive correlations were observed among nitrification, denitrification, and dissimilatory nitrate reduction genes (p-adjusted < 0.05, $\rho > 0.9$) in N fertilizer applied soils. Only correlation between nitrification and dissimilatory nitrate reduction existed in control (p-adjusted < 0.05, $\rho >$ 0.9) (Fig. 5.5). The results indicate that inorganic N application may stimulate N loss through strength the correlation of gene abundances involved in nitrification and denitrification, denitrification, and dissimilatory nitrate reduction process.

5.3.3 Viral genes related to carbohydrates metabolism

We found 4 non-redundant auxiliary metabolic genes (AMGs) of viruses proposed to participate in starch and sucrose metabolism (glycogen synthase), galactose metabolism (UDP-galactopyranose mutase, *glf*; UDP-glucose 4-epimerase, *galE*), fructose and mannose metabolism (GDP-mannose 4,6-dehydratase, *gmd*) (Fig. 5.6 and Table S5.5). The *galE* and *glf* AMGs were only identified in the no cover treatment. Glycogen synthase genes were found in vetch without fertilization management, and *gmd* was in all treatments (Fig. 6 and Table S5). However, *galE*, *glf*, glycogen synthase only can be identified in one sample out of three replicates for each treatment. The possible reasons may relate to sequencing depth, heterogeneity of soil samples, and AMGs with significant abundant in specific plot or treatment.

Phylogenetic trees based upon protein sequences of AMGs, and protein sequences encoding by microbial genes obtained in RefSeq database were used to examine origination of each AMGs. AMGs of glycogen synthase were confidently clustered with *Cyanobacteria* (*Synechococcaceae*) (Fig. 5.7A). AMG of *gmd* and same gene in *Actinobacteria Streptomyces* were homologous (Fig. 5.7B). The *glf* were classified within the lineage of *Planctomycetes Blastopirellula* (Fig. 5.6C). The *galE* was found in three vOTUs, vOTU_119, vOTU_85, and vOTU_46 (Table S5.5). AMG from vOTU_85 and vOTU_119 was clustered with *Actinobacteria*, AMG from vOTU_46 was in *Proteobacteria* lineages (Fig. 5.7D). Our results described above indicate that viruses may influence the performance of *Cyanobacteria*, *Planctomycetes*, *Proteobacteria*, and *Actinobacteria* in C cycling during the infection through AMGs mediated microbial C metabolism.

5.4 Discussion

Our objective for this study was to understand how variations of microbial potential function respond to variations of soil environment induced by agricultural management, which is crucial for predicting the soil function under the climate change and feedbacks (Xue et al., 2016). In this study, not only alterations in soil microbial genes related to C compounds metabolism and N were observed under fertilization and cover cropping management via differential abundance analysis and redundancy analysis, but the evidence of viral gene associate microbial carbohydrate metabolism was found by phylogenetic analysis through DNA-based metagenome technique.

5.4.1 The influence of management on gene for C decomposition

Inorganic N fertilizer application more significantly influenced C degradation gene abundance than cover cropping. Genes for degrading more chemically recalcitrant C components (i.e., pectin, chitin, and lignin) increased under no fertilization treatments compared with the soil under with fertilization treatments. N addition tends to accelerate the degradation of labile organic C component (decades of turnover times) than the heavier C fractions (multidecadal to century lifetimes) (Neff et al., 2002). Excess exogenous N supply could dampen microbial nitrogen mining from recalcitrant C substrates, reducing the decay rate of recalcitrant C and lead to increasing C storage in the long-term (Magill & Aber, 1998; Hagedorn et al., 2003; Craine et al., 2007). The lower abundance of genes coding for decomposition of chemically recalcitrant C in N fertilizer treated plots in our study support this theory.

Conversely, when N is limiting, microbes may utilize labile C to decompose recalcitrant organic matter to obtain N (Moorhead & Sinsabaugh, 2006; Craine et al., 2007). When the soil inorganic N was limited, abundances of genes encoding for starch, hemicellulose and lignin degradation increased in treatments with no direct inorganic N input. beta-N-acetylglucosaminidase (NAGase) is a key gene for catabolizing chitin and releasing NAGases can release β-1, 4-linked N-acetyl-d-glucosamine (GlcNAc) monosaccharide from chitin, which can represent a significant resources of N for microbes (Roberts et al., 2007; Beier & Bertilsson, 2013; Du et al., 2019). The abundances of genes encoding beta-N-acetylglucosaminidase (NAGase) also increased in no fertilization treatment or no vetch covered compared with vetch with fertilization,

indicating that may still acquire N from available organic compounds in single N resource (N fertilization or vetch cover) than multisource (N fertilization and vetch cover).

5.4.2 The influence of management on N-cycling gene

Continuous N fertilizer application cab increase soil fertility and crop productivity (Mbuthia et al., 2015b), and enhance nitrification and denitrification (Chen et al., 2012); two processes which accelerate removal of reactive N as N_2, N2O, NO (Skiba & Smith, 1993; Abbasi & Adams, 2000; Ouyang et al., 2018; Wang et al., 2018a). In our study, we observed that abundances of *amoC* and *norB* increased under fertilization indicating that nitrification and denitrification has been enhanced. Meanwhile, dissimilatory nitrate reduction gene (*nirB*) was abundant in fertilization treatments and control, and dissimilatory nitrate reduction which was a pathway for N retention was positively correlated with denitrification (Pandey et al., 2019). Our results suggest that an equilibrium between N loss through denitrification and N retention may occurring under inorganic N addition. In addition, no fertilizer treated soil may through DRNA to conserve N, which consist with previous findings, DRNA process was dominated and explained a large amount of nitrate in long-term low N treated soil (Pandey et al., 2019). Above all, our results indicated that microbial N cycling strategies have evolved to maintain soil N nutrition in a long-term in inorganic N input soil.

5.4.3 The function of viral AMGs in microbes and its role in C metabolism

AMGs identified in our study may potentially influence microbial glycolysis, including: synthesis of polysaccharide, lipopolysaccharide (LPS) and extracellular polymeric substances (EPS). The UDP-glucose 4-epimerase (*galE*) encoded enzyme responsible for reversible epimerization of UDP-glucose to UDP-galactose involved in Leloir pathway for galactose metabolism in microbes (Paladini & Leloir, 1952; Holden et al., 2003; Kim et al., 2011). This reaction would influence the content of UDP-glucose. In the aspects of catabolism, UDP-glucose is the precursor for glucose-6-phosphate (Thoden & Holden, 2007). Glucose-6-phosphate associate glycolysis and influence the energy generation in microbes (Lucarelli et al., 2015). In the aspects of anabolism, UDP-glucose not only as the substrate involved in glycogen synthesis, but it act as a glycosyl donor and connect to carbohydrates, proteins, lipids, DNA, and other small molecules, which important in biosynthesis pathways of oligosaccharides and polysaccharides, as well as protein glycosylation. This would benefit for viral capsid assembly and reproduction during the lysis (Schmid et al., 2016; Breitbart et al., 2018).

Prophage might directly improve the host fitness and enhance host metabolism or extend the pathways for the host that cannot store energy from low nutrients, unfavorable environmental conditions. (Strange, 1968; Edlin et al., 1975; Edlin et al., 1977; Paul, 2008; Sekulovic & Fortier, 2015; Obeng et al., 2016; Breitbart et al., 2018). Glycogen synthase gene was found in *Cyanobacteria* phyla have also been reported in a previous study (Yoo et al., 2014). Glycogen is an important form of energy storage in many microbes since it can be further decomposed and produce alpha-D-glucose-1-phosphate to involve in glycolysis for energy generation in bacteria

(Wilson et al., 2010) (Suzuki et al., 2010; Shinde et al., 2020). We identified genes encoding glycogen synthase on identified vOTUs, indicating that some viral infections can redistribute carbon via non-glycolytic pathways by triggering starvation responses in their hosts (Hurwitz et al., 2013).

Moreover, the products of UDP-galactopyranose mutase, UDP-galactofuranose (UDP-Galf) residues are lipopolysaccharide (LPS) O-antigens that exist in many Gram-negative bacteria (Besra et al., 1995; Pan et al., 2001; Sanders et al., 2001). GDP-4-keto-6-deoxy-d-mannose is the product of GDP-D-mannose 4,6-dehydratase (*gmd)*, which can be used for biosynthesis of fucose, rhamnose, and 6-deoxy-talose, which are important 6-deoxyhexoses for lipopolysaccharides (LPSs), extracellular polysaccharides (EPSs), glycoproteins on bacteria (Somoza et al., 2000; Mäki & Renkonen, 2004). AMGs involved in those microbial polysaccharide compounds formation may be important labile and recalcitrant C resources in soil (Martin, 1971; Kikuchi & Komatsu, 2017).

Prophages can participate in biofilm formation reported for groups such as *Firmicutes* and *Proteobacteria* (Rice et al., 2009; Schuch & Fischetti, 2009). UDP-glucose and UDP-galactose are essential components of EPS biofilm formation in bacteria (Chai et al., 2012). Downregulation or depletion of *galE* mediated by virus might lead to rapid bacterial cell lysis due to accumulation of toxic galactose metabolites (UDP-galactose) in the bacterial host. This importance of *galE* has been verified in *Bacillus subtilis* strains (Krispin & Allmansberger, 1998; Chai et al., 2012). Conversely, if the phage could increase the *galE* translation, it might promote the formation of exopolysaccharide (EPS) component of the biofilm matrix. Viral particles can

use the microbial biofilm as protection and survive from desiccation and other environmental stress (Vasickova et al., 2010). After the erosion of biofilms, viral particles would be released and target its host and complete reproduction (Lacroix-Gueu et al., 2005; Briandet et al., 2008; Helmi et al., 2008).

All the AMGs show important roles in microbial C metabolism in this study. However, we did not find genes from viruses directly involved in microbial central carbon metabolism (including glycolysis, pentose phosphate pathway, and citric acid cycle), sporulation in C metabolism or gene related to N-cycling (Emerson et al., 2018; Trubl et al., 2018a). Our results who that phage have the ability to manipulate the host metabolism to build a defense mechanism (host biofilm formation, energy storage) for themselves and hosts from harsh environment in the soil.

Collectively, drawing information of AMGs from bulk soil metagenomes give limited, but significant, knowledge of viral functional influence on microbial communities and C and N cycling. This might be due to the difficulties in reaching to enough sequencing depth on environmental soil samples. Also, the heterogeneity of soil brings in strong background noise from microbial sequences and increased the challenge of explore viral function in agroecosystem (Jansson & Hofmockel, 2018; Trubl et al., 2018a; Trubl et al., 2020). We might benefit more from the viral information acquired from soil viromes, or specific virus-host linkage in laboratory experiments when studying the viral communities and mechanism between virus and its host in future.

5.5 Conclusions

Inorganic N fertilization appears to promote relatively labile C substrate decomposition by increasing abundances of genes involved in decomposing hemicellulose and cellulose. Pectin and chitin decomposition genes increased in abundance under no cover cropping treatment compared with vetch covered soil. This indicates that N fertilization and cover cropping have positive effect on soil C storage by providing N and readily C resources. And restrictions on N and C input may redistributed soil organic C pool and dampen soil recalcitrant C pool.

The structure of microbial C degradation and N-cycling genes across samples were strongly influenced by inorganic N fertilization compared to cover cropping in over 40-year continuous cotton plantation system. Inorganic N fertilization, more than cover cropping, appeared to increase nitrification, denitrification, dissimilatory nitrate reduction gene abundance. Significantly positive correlations gene relative abundances between dissimilatory nitrate reduction, denitrification processes were found under both N fertilizer addition and no fertilizer treated soil, suggesting that microbes developed the strategy to conserve N to maintain N nutrient levels via dissimilatory nitrate reduction.

In addition, AMGs identified from viruses may plays important roles in microbial energy production and storage, receptor recognition, etc. Those genes may originate from *Cyanobacteria*, *Planctomycetes*, *Proteobacteria*, and *Actinobacteria* phyla. This study provides crucial information on how soil microbes C degradation and N-cycling genes response field managements and reveals the importance of soil viruses on influencing microbial C metabolism

through AMGs. This would help to sustain soil N and C resources and prolong the soil fertility and productivity from a more complete interaction between microbes-virus perspective.

References

Abbasi, M., Adams, W. 2000. Gaseous N emission during simultaneous nitrification–denitrification associated with mineral N fertilization to a grassland soil under field conditions. Soil Biology and Biochemistry, 32(8-9), 1251-1259.

Acosta-Martínez, V., Burow, G., Zobeck, T., Allen, V. 2010. Soil microbial communities and function in alternative systems to continuous cotton. Soil Science Society of America Journal, 74(4), 1181-1192.

Ashelford, K. E., Day, M. J., Fry, J. C. 2003. Elevated abundance of bacteriophage infecting bacteria in soil. Applied and environmental microbiology, 69(1), 285-289.

Averill, C., Waring, B. 2018. Nitrogen limitation of decomposition and decay: How can it occur? Global Change Biology, 24(4), 1417-1427.

Bacon, J. (1968). The Chemical Envir-onments of bacteria in soil. In the Ecology of Soil Bacteria. In: Liverpool University press.

Batjes, N. H. 2014. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science, 65(1), 10-21.

Beier, S., Bertilsson, S. 2013. Bacterial chitin degradation—mechanisms and ecophysiological strategies. Frontiers in Microbiology, 4, 149.

Benjamini, Y., Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological), 57(1), 289-300.

Besra, G. S., Khoo, K.-H., McNeil, M. R., Dell, A., Morris, H. R., Brennan, P. J. 1995. A new interpretation of the structure of the mycolyl-arabinogalactan complex of Mycobacterium tuberculosis as revealed through characterization of oligoglycosylalditol fragments by

fast-atom bombardment mass spectrometry and 1H nuclear magnetic resonance spectroscopy. Biochemistry, 34(13), 4257-4266.

Breitbart, M., Bonnain, C., Malki, K., Sawaya, N. A. 2018. Phage puppet masters of the marine microbial realm. Nature Microbiology, 3(7), 754-766.

Briandet, R., Lacroix-Gueu, P., Renault, M., Lecart, S., Meylheuc, T., Bidnenko, E., Steenkeste, K., Bellon-Fontaine, M.-N., Fontaine-Aupart, M.-P. 2008. Fluorescence correlation spectroscopy to study diffusion and reaction of bacteriophages inside biofilms. Applied and environmental microbiology, 74(7), 2135-2143.

Chai, Y., Beauregard, P. B., Vlamakis, H., Losick, R., Kolter, R. 2012. Galactose metabolism plays a crucial role in biofilm formation by Bacillus subtilis. MBio, 3(4), e00184-00112.

Che, R., Qin, J., Tahmasbian, I., Wang, F., Zhou, S., Xu, Z., Cui, X. 2018. Litter amendment rather than phosphorus can dramatically change inorganic nitrogen pools in a degraded grassland soil by affecting nitrogen-cycling microbes. Soil Biology and Biochemistry, 120, 145-152.

Chen, Z., Liu, J., Wu, M., Xie, X., Wu, J., Wei, W. 2012. Differentiated response of denitrifying communities to fertilization regime in paddy soil. Microbial Ecology, 63(2), 446-459.

Cheshire, M. (1985). Carbohydrates in relation to soil fertility. In *Soil organic matter and biological activity* (pp. 263-288): Springer.

Craine, J. M., Morrow, C., Fierer, N. 2007. Microbial nitrogen limitation increases decomposition. Ecology, 88(8), 2105-2113.

Crummett, L. T., Puxty, R. J., Weihe, C., Marston, M. F., Martiny, J. B. 2016. The genomic content and context of auxiliary metabolic genes in marine cyanomyoviruses. Virology, 499, 219-229.

Du, C., Jiang, S., Jiang, S., Zhou, Y., Zhang, G. 2019. A Bacillus pumilus originated β-N-acetylglucosaminidase for chitin combinatory hydrolysis and exploration of its thermostable mechanism. International journal of biological macromolecules, 132, 1282-1289.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research, 32(5), 1792-1797.

Edlin, G., Lin, L., Bitner, R. 1977. Reproductive fitness of P1, P2, and Mu lysogens of Escherichia coli. Journal of virology, 21(2), 560-564.

Edlin, G., Lin, L., Kudrna, R. 1975. λ lysogens of E. coli reproduce more rapidly than non-lysogens. Nature, 255(5511), 735-737.

Emerson, J. B., Roux, S., Brum, J. R., Bolduc, B., Woodcroft, B. J., Jang, H. B., Singleton, C. M., Solden, L. M., Naas, A. E., Boyd, J. A. 2018. Host-linked soil viral ecology along a permafrost thaw gradient. Nature Microbiology, 3(8), 870-880.

Enav, H., Mandel-Gutfreund, Y., Béjà, O. 2014. Comparative metagenomic analyses reveal viral-induced shifts of host metabolism towards nucleotide biosynthesis. Microbiome, 2(1), 1-12.

Evans, J. T., Denef, V. J. 2020. To dereplicate or not to dereplicate? Msphere, 5(3).

Graham, E. B., Paez-Espino, D., Brislawn, C., Neches, R. Y., Hofmockel, K. S., Wu, R., Kyrpides, N. C., Jansson, J. K., McDermott, J. E. 2019. Untapped viral diversity in global soil metagenomes. bioRxiv, 583997.

Hagedorn, F., Spinnler, D., Siegwolf, R. 2003. Increased N deposition retards mineralization of old soil organic matter. Soil Biology and Biochemistry, 35(12), 1683-1692.

Helmi, K., Skraber, S., Gantzer, C., Willame, R., Hoffmann, L., Cauchie, H.-M. 2008. Interactions of Cryptosporidium parvum, Giardia lamblia, vaccinal poliovirus type 1, and bacteriophages ΦX174 and MS2 with a drinking water biofilm and a wastewater biofilm. Applied and environmental microbiology, 74(7), 2079-2088.

Holden, H. M., Rayment, I., Thoden, J. B. 2003. Structure and function of enzymes of the Leloir pathway for galactose metabolism. Journal of Biological Chemistry, 278(45), 43885-43888.

Homyak, P. M., Allison, S. D., Huxman, T. E., Goulden, M. L., Treseder, K. K. 2017. Effects of drought manipulation on soil nitrogen cycling: A meta-analysis. Journal of Geophysical Research: Biogeosciences, 122(12), 3260-3272.

Huang, X., Jiao, N., Zhang, R. 2021. The genomic content and context of auxiliary metabolic genes in roseophages. Environmental microbiology.

Hurwitz, B. L., Hallam, S. J., Sullivan, M. B. 2013. Metabolic reprogramming by viruses in the sunlit and dark ocean. Genome biology, 14(11), 1-14.

Jansson, J. K., Hofmockel, K. S. 2018. The soil microbiome—from metagenomics to metaphenomics. Current opinion in microbiology, 43, 162-168.

Jian, S., Li, J., Chen, J., Wang, G., Mayes, M. A., Dzantor, K. E., Hui, D., Luo, Y. 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biology and Biochemistry, 101, 32-43.

Kikuchi, J., Komatsu, T. (2017). Polysaccharides as major carbon sources in environmental biodiversity. In *NMR in Glycoscience and Glycotechnology* (pp. 369-395).

Kim, H.-J., Kang, S. Y., Park, J. J., Kim, P. 2011. Novel activity of UDP-galactose-4-epimerase for free monosaccharide and activity improvement by active site-saturation mutagenesis. Applied biochemistry and biotechnology, 163(3), 444-451.

Kraft, B., Strous, M., Tegetmeyer, H. E. 2011. Microbial nitrate respiration–genes, enzymes and environmental distribution. Journal of biotechnology, 155(1), 104-117.

Krispin, O., Allmansberger, R. 1998. The Bacillus subtilis galE gene is essential in the presence of glucose and galactose. Journal of bacteriology, 180(8), 2265-2270.

Lacroix-Gueu, P., Briandet, R., Lévêque-Fort, S., Bellon-Fontaine, M.-N., Fontaine-Aupart, M.-P. 2005. In situ measurements of viral particles diffusion inside mucoid biofilms. Comptes rendus biologies, 328(12), 1065-1072.

LeBauer, D. S., Treseder, K. K. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology, 89(2), 371-379.

Li, D., Liu, C.-M., Luo, R., Sadakane, K., Lam, T.-W. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics, 31(10), 1674-1676.

Li, D., Luo, R., Liu, C.-M., Leung, C.-M., Ting, H.-F., Sadakane, K., Yamashita, H., Lam, T.-W. 2016. MEGAHIT v1. 0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods, 102, 3-11.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics, 25(16), 2078-2079.

Li, W., Godzik, A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics, 22(13), 1658-1659.

Li, W., Jaroszewski, L., Godzik, A. 2002. Tolerating some redundancy significantly speeds up clustering of large protein databases. Bioinformatics, 18(1), 77-82.

Liang, C., Schimel, J. P., Jastrow, J. D. 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology, 2(8), 1-6.

Love, M. I., Huber, W., Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology, 15(12), 1-21.

Lucarelli, G., Galleggiante, V., Rutigliano, M., Sanguedolce, F., Cagiano, S., Bufo, P., Lastilla, G., Maiorano, E., Ribatti, D., Giglio, A. 2015. Metabolomic profile of glycolysis and the pentose phosphate pathway identifies the central role of glucose-6-phosphate dehydrogenase in clear cell-renal cell carcinoma. Oncotarget, 6(15), 13371.

Magill, A. H., Aber, J. D. 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant and Soil, 203(2), 301-311.

Mäki, M., Renkonen, R. 2004. Biosynthesis of 6-deoxyhexose glycans in bacteria. Glycobiology, 14(3), 1R-15R.

Martin, J. P. 1971. Decomposition and binding action of polysaccharides in soil. Soil Biology and Biochemistry, 3(1), 33-41.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal, 17(1), 10-12.

Martínez-García, L. B., Korthals, G., Brussaard, L., Jørgensen, H. B., De Deyn, G. B. 2018. Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. Agriculture, Ecosystems & Environment, 263, 7-17.

Mbuthia, L. W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N. 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biology and Biochemistry, 89, 24-34.

Mikheenko, A., Prjibelski, A., Saveliev, V., Antipov, D., Gurevich, A. 2018. Versatile genome assembly evaluation with QUAST-LG. Bioinformatics, 34(13), i142-i150.

Moorhead, D. L., Sinsabaugh, R. L. 2006. A theoretical model of litter decay and microbial interaction. Ecological monographs, 76(2), 151-174.

Neff, J. C., Townsend, A. R., Gleixner, G., Lehman, S. J., Turnbull, J., Bowman, W. D. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature, 419(6910), 915-917.

Norton, J. M., Stark, J. M. 2011. Regulation and measurement of nitrification in terrestrial systems. Methods in enzymology, 486, 343-368.

Obeng, N., Pratama, A. A., van Elsas, J. D. 2016. The significance of mutualistic phages for bacterial ecology and evolution. Trends in microbiology, 24(6), 440-449.

Ouyang, Y., Evans, S. E., Friesen, M. L., Tiemann, L. K. 2018. Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: a meta-analysis of field studies. Soil Biology and Biochemistry, 127, 71-78.

Paladini, A. C., Leloir, L. 1952. Studies on uridine-diphosphate-glucose. Biochemical Journal, 51(3), 426.

Pan, F., Jackson, M., Ma, Y., McNeil, M. 2001. Cell wall core galactofuran synthesis is essential for growth of mycobacteria. Journal of bacteriology, 183(13), 3991-3998.

Pandey, A., Suter, H., He, J.-Z., Hu, H.-W., Chen, D. 2019. Dissimilatory nitrate reduction to ammonium dominates nitrate reduction in long-term low nitrogen fertilized rice paddies. Soil Biology and Biochemistry, 131, 149-156.

Paul, J. H. 2008. Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? The ISME journal, 2(6), 579-589.

Paustian, K., Larson, E., Kent, J., Marx, E., Swan, A. 2019. Soil C sequestration as a biological negative emission strategy. Frontiers in Climate, 1, 8.

Philippot, L., Hallin, S., Schloter, M. 2007. Ecology of denitrifying prokaryotes in agricultural soil. Advances in Agronomy, 96, 249-305.

Prescott, C. E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry, 101(1), 133-149.

Price, M. N., Dehal, P. S., Arkin, A. P. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Molecular biology and evolution, 26(7), 1641-1650.

Quinlan, A. R., Hall, I. M. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics, 26(6), 841-842.

Raun, W., Johnson, G., Phillips, S., Westerman, R. 1998. Effect of long-term N fertilization on soil organic C and total N in continuous wheat under conventional tillage in Oklahoma. Soil and Tillage Research, 47(3-4), 323-330.

Rice, S. A., Tan, C. H., Mikkelsen, P. J., Kung, V., Woo, J., Tay, M., Hauser, A., McDougald, D., Webb, J. S., Kjelleberg, S. 2009. The biofilm life cycle and virulence of Pseudomonas aeruginosa are dependent on a filamentous prophage. The ISME journal, 3(3), 271-282.

Roberts, P., Bol, R., Jones, D. L. 2007. Free amino sugar reactions in soil in relation to soil carbon and nitrogen cycling. Soil Biology and Biochemistry, 39(12), 3081-3092.

Salinas-Garcia, J., Hons, F., Matocha, J., Zuberer, D. 1997. Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization. Biology and Fertility of Soils, 25(2), 182-188.

Sanders, D. A., Staines, A. G., McMahon, S. A., McNeil, M. R., Whitfield, C., Naismith, J. H. 2001. UDP-galactopyranose mutase has a novel structure and mechanism. Nature structural biology, 8(10), 858-863.

Schmid, J., Heider, D., Wendel, N. J., Sperl, N., Sieber, V. 2016. Bacterial glycosyltransferases: challenges and opportunities of a highly diverse enzyme class toward tailoring natural products. Frontiers in Microbiology, 7, 182.

Schuch, R., Fischetti, V. A. 2009. The secret life of the anthrax agent Bacillus anthracis: bacteriophage-mediated ecological adaptations. PLoS One, 4(8), e6532.

Sekulovic, O., Fortier, L.-C. 2015. Global transcriptional response of Clostridium difficile carrying the ϕCD38-2 prophage. Applied and environmental microbiology, 81(4), 1364-1374.

Shinde, S., Zhang, X., Singapuri, S. P., Kalra, I., Liu, X., Morgan-Kiss, R. M., Wang, X. 2020. Glycogen metabolism supports photosynthesis start through the oxidative pentose phosphate pathway in cyanobacteria. Plant physiology, 182(1), 507-517.

Skiba, U., Smith, K. 1993. Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. Soil Biology and Biochemistry, 25(11), 1527-1536.

Smith, J. 1966. Some inter-relationships between decomposition of various plant residues and loss of soil organic matter as measured by carbon-14 labelling.

Somoza, J. R., Menon, S., Schmidt, H., Joseph-McCarthy, D., Dessen, A., Stahl, M. L., Somers, W. S., Sullivan, F. X. 2000. Structural and kinetic analysis of Escherichia coli GDP-mannose 4, 6 dehydratase provides insights into the enzyme's catalytic mechanism and regulation by GDP-fucose. Structure, 8(2), 123-135.

Starr, E. P., Nuccio, E. E., Pett-Ridge, J., Banfield, J. F., Firestone, M. K. 2019. Metatranscriptomic reconstruction reveals RNA viruses with the potential to shape carbon cycling in soil. Proceedings of the National Academy of Sciences, 116(51), 25900-25908.

Strange, R. 1968. Bacterial "glycogen" and survival. Nature, 220(5167), 606-607.

Sun, S., Badgley, B. D. 2019. Changes in microbial functional genes within the soil metagenome during forest ecosystem restoration. Soil Biology and Biochemistry, 135, 163-172.

Suzuki, E., Ohkawa, H., Moriya, K., Matsubara, T., Nagaike, Y., Iwasaki, I., Fujiwara, S., Tsuzuki, M., Nakamura, Y. 2010. Carbohydrate metabolism in mutants of the cyanobacterium Synechococcus elongatus PCC 7942 defective in glycogen synthesis. Applied and environmental microbiology, 76(10), 3153-3159.

ter Horst, A. M., Santos-Medellín, C., Sorensen, J. W., Zinke, L. A., Wilson, R. M., Johnston, E. R., Trubl, G. G., Pett-Ridge, J., Blazewicz, S. J., Hanson, P. J. 2020. Minnesota peat viromes reveal terrestrial and aquatic niche partitioning for local and global viral populations. bioRxiv.

Thamdrup, B. 2012. New pathways and processes in the global nitrogen cycle. Annual Review of Ecology, Evolution, and Systematics, 43, 407-428.

Thoden, J. B., Holden, H. M. 2007. The molecular architecture of glucose-1-phosphate uridylyltransferase. Protein science, 16(3), 432-440.

Thompson, L. R., Zeng, Q., Kelly, L., Huang, K. H., Singer, A. U., Stubbe, J., Chisholm, S. W. 2011. Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. Proceedings of the National Academy of Sciences, 108(39), E757-E764.

Trubl, G., Hyman, P., Roux, S., Abedon, S. T. 2020. Coming-of-age characterization of soil viruses: A user's guide to virus isolation, detection within metagenomes, and viromics. Soil Systems, 4(2), 23.

Trubl, G., Jang, H. B., Roux, S., Emerson, J. B., Solonenko, N., Vik, D. R., Solden, L., Ellenbogen, J., Runyon, A. T., Bolduc, B. 2018. Soil viruses are underexplored players in ecosystem carbon processing. Msystems, 3(5).

Vasickova, P., Pavlik, I., Verani, M., Carducci, A. 2010. Issues concerning survival of viruses on surfaces. Food and Environmental Virology, 2(1), 24-34.

Wang, F., Chen, S., Wang, Y., Zhang, Y., Hu, C., Liu, B. 2018. Long-term nitrogen fertilization elevates the activity and abundance of nitrifying and denitrifying microbial communities in an upland soil: implications for nitrogen loss from intensive agricultural systems. Frontiers in Microbiology, 9, 2424.

Williamson, K. E., Fuhrmann, J. J., Wommack, K. E., Radosevich, M. 2017. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. Annual review of virology, 4, 201-219.

Wilson, W. A., Roach, P. J., Montero, M., Baroja-Fernández, E., Muñoz, F. J., Eydallin, G., Viale, A. M., Pozueta-Romero, J. 2010. Regulation of glycogen metabolism in yeast and bacteria. FEMS microbiology reviews, 34(6), 952-985.

Wortman, S. E., Drijber, R. A., Francis, C. A., Lindquist, J. L. 2013. Arable weeds, cover crops, and tillage drive soil microbial community composition in organic cropping systems. Applied Soil Ecology, 72, 232-241.

Xue, K., Xie, J., Zhou, A., Liu, F., Li, D., Wu, L., Deng, Y., He, Z., Van Nostrand, J. D., Luo, Y. 2016. Warming alters expressions of microbial functional genes important to ecosystem functioning. Frontiers in Microbiology, 7, 668.

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., Zhang, Y. 2015. The I-TASSER Suite: protein structure and function prediction. Nature Methods, 12(1), 7-8.

Yoo, S.-H., Lee, B.-H., Moon, Y., Spalding, M. H., Jane, J.-l. 2014. Glycogen synthase isoforms in Synechocystis sp. PCC6803: identification of different roles to produce glycogen by targeted mutagenesis. PLoS One, 9(3), e91524.

Yuan, Z., Chen, H. Y. 2012. A global analysis of fine root production as affected by soil nitrogen and phosphorus. Proceedings of the Royal Society B: Biological Sciences, 279(1743), 3796-3802.

Appendix 5

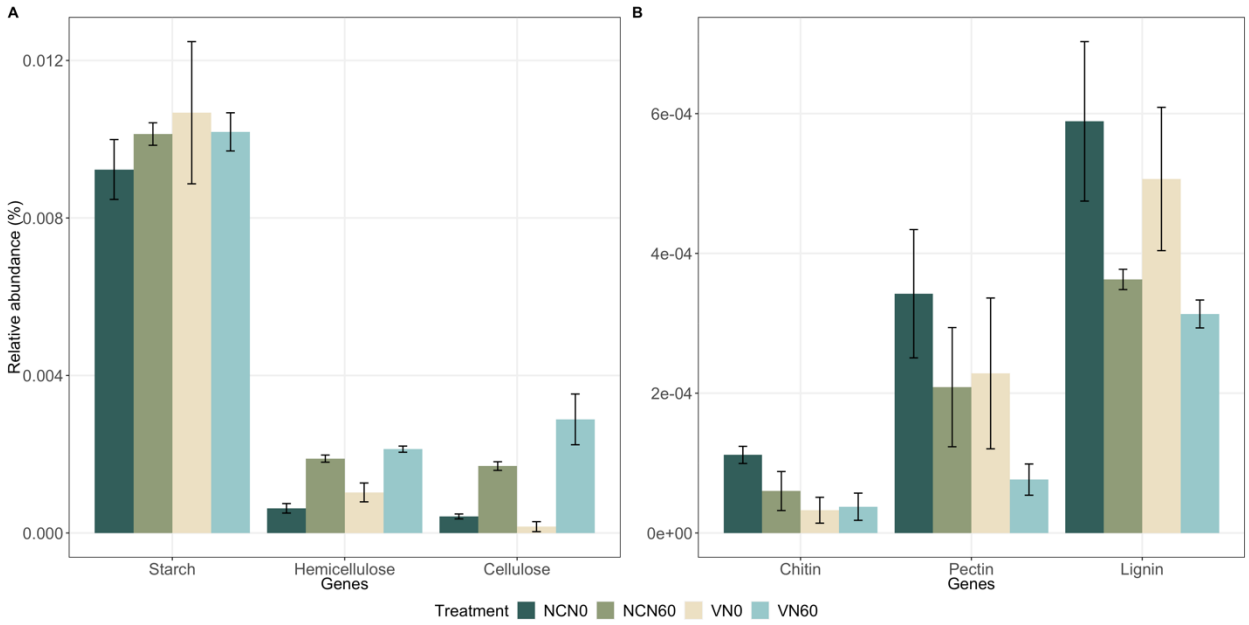


Figure 5.1 Gene relative abundances by decomposition of different C substrate.

Starch, hemicellulose, cellulose (Fig. 5.1A), chitin, pectin, and lignin (Fig. 5.1B) are listed in order from chemically labile to recalcitrant. The influence of N fertilization and cover cropping on gene abundances were shown in 1A and 1B, 1C and 1D, respectively. Abundance of genes for each category were normalized using DESeq2. Gene abundances related to hemicellulose ($p < 0.001$) and cellulose ($p < 0.001$) were significantly greater in fertilization than control. However, genes abundances for decomposing lignin ($p < 0.01$) decreased in fertilization. Hemicellulose ($p < 0.05$), pectin ($p < 0.05$), and chitin ($p < 0.05$) were significantly greater in no-cover treated than vetch covered plots. Cover cropping and fertilization had no effect on relative abundances of genes related to starch decomposition. No interaction effect between two treatments among different C substrates can be observed. Error bars represent the standard error of the mean (n=3).

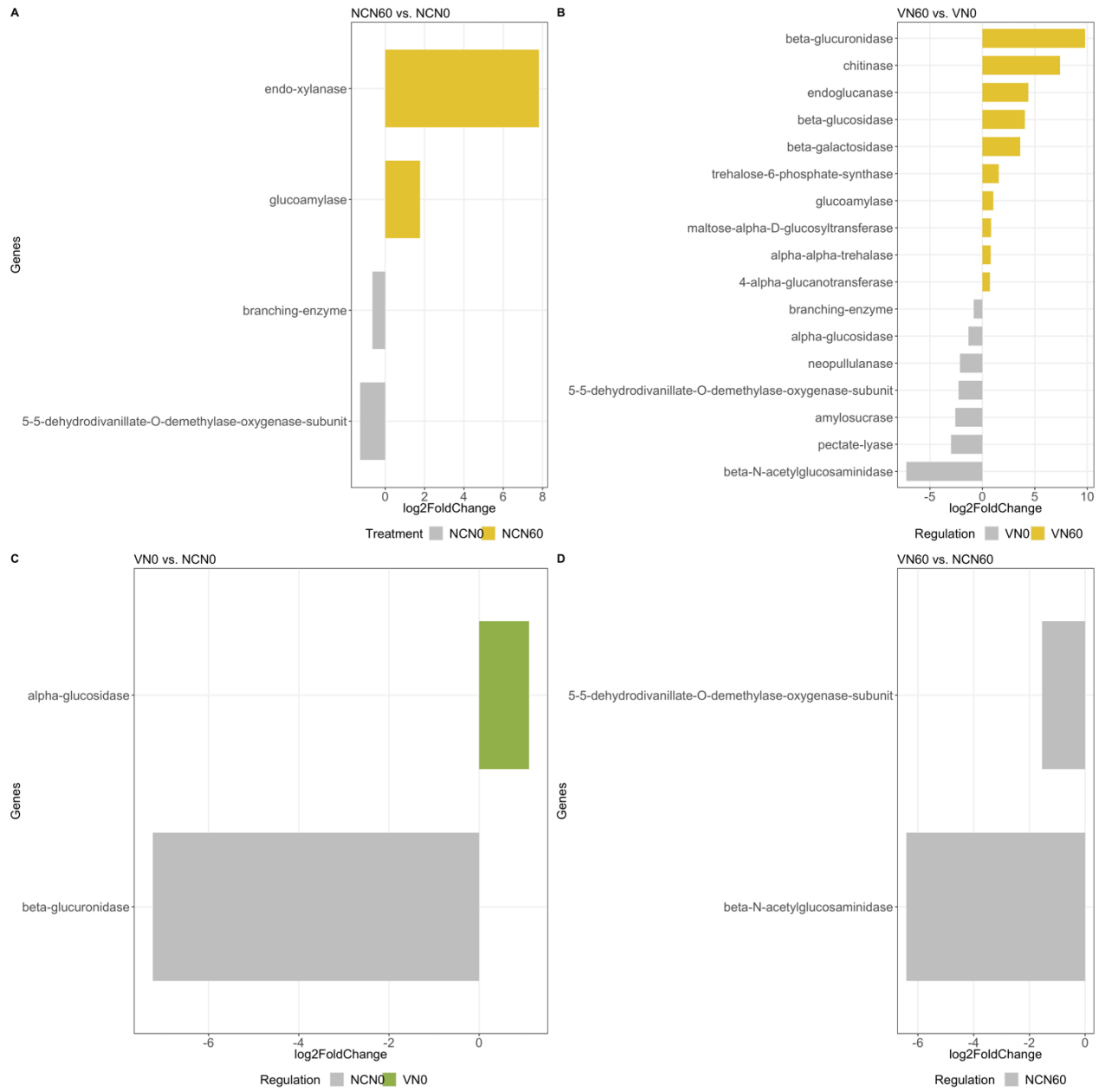


Figure 5.2 Differential abundances analysis on microbial gene for carbohydrate decomposition.

Bar plots represent the log2FoldChange according to DESeq2 analysis (FDR < 0.1). Positive value means that the genes were significantly more abundant in NCN60 (A), VN60 (B), and

VN0 (C). Negative value represented genes contain significantly greater abundance in NCN0 (A), VN0 (B), NCN0 (C), and NCN60 (D) than the other treatment in the same figures.

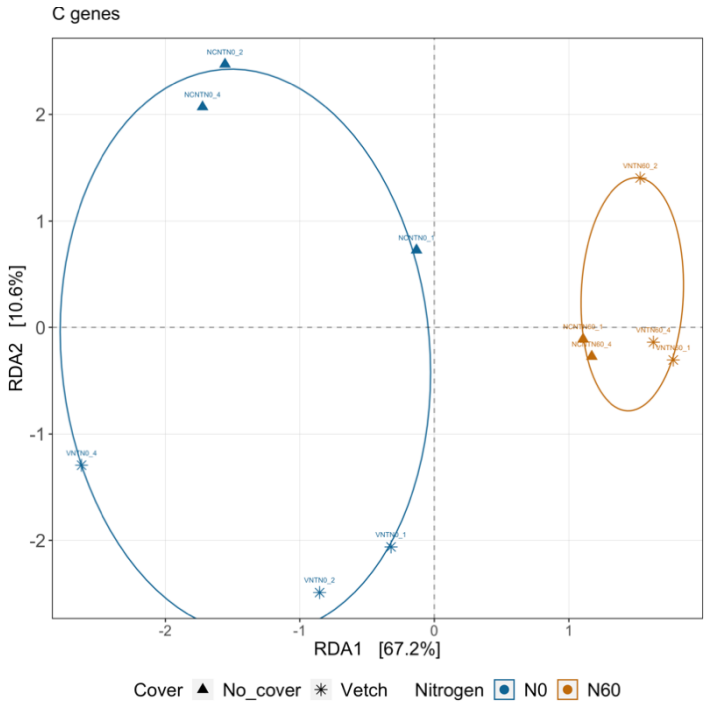


Figure 5.3 Redundancy analysis on microbial C decomposition gene.

Abundance of C decomposition gene in each sample were normalized by DESeq2.

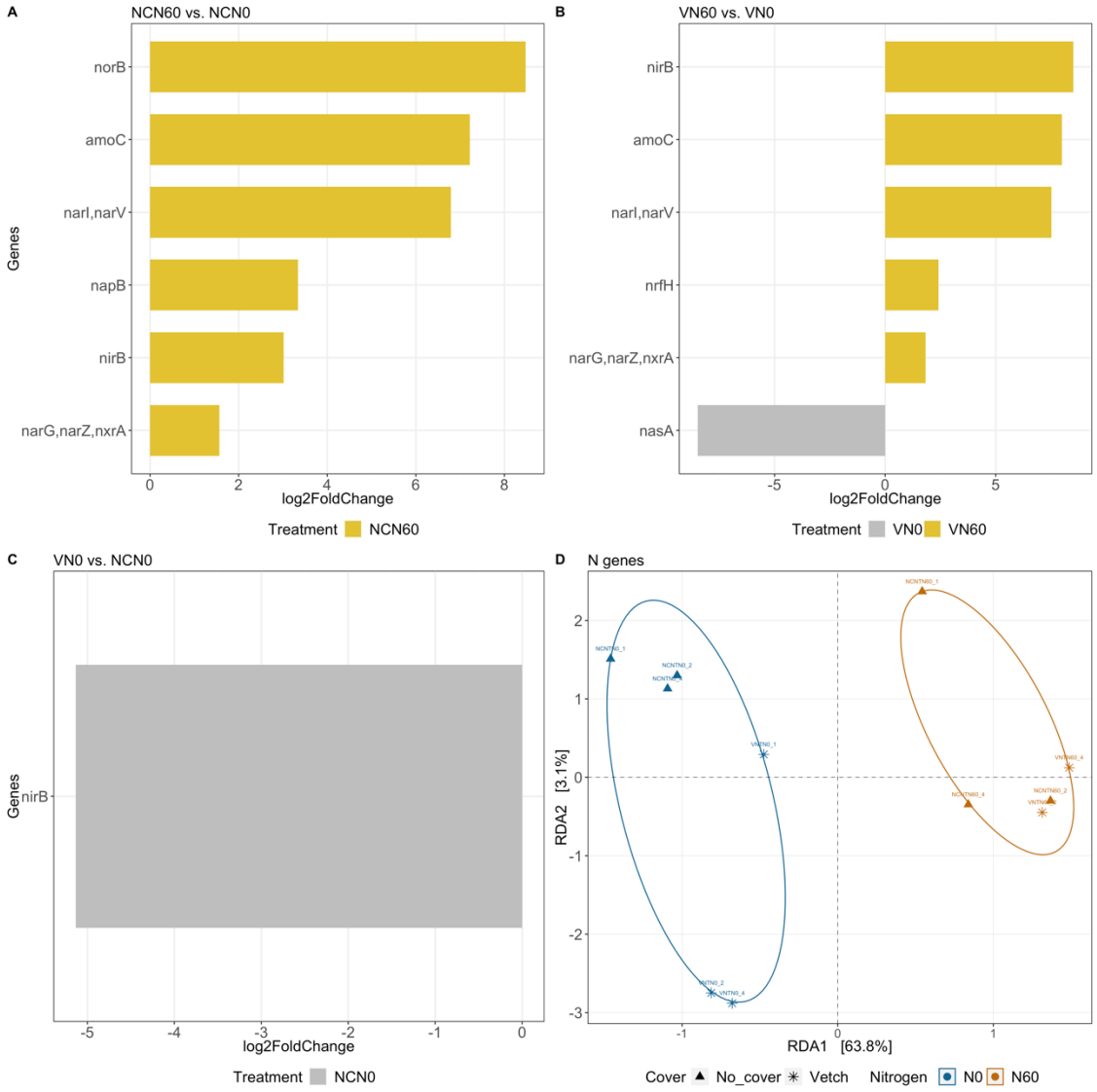


Figure 5.4 Differential abundance analysis on N-cycling gene of microbial communities.

Bar plots represent the log2FoldChange according to DESeq2 analysis (FDR < 0.1 Positive value means that the genes were significantly more abundant in NCN60 (A), VN60 (B), and VN0 (C). Negative value represented genes have significantly greater abundance in NCN0 (A), VN0 (B), NCN0 (C) than the other treatment in the same figures (i.e., *nasA* were significantly abundant in

no-fertilized soil than fertilized soil). No gene was significantly different between VN60 and NCN60. Redundancy analysis (RDA) was applied on the gene counts of each sample after normalization (D).

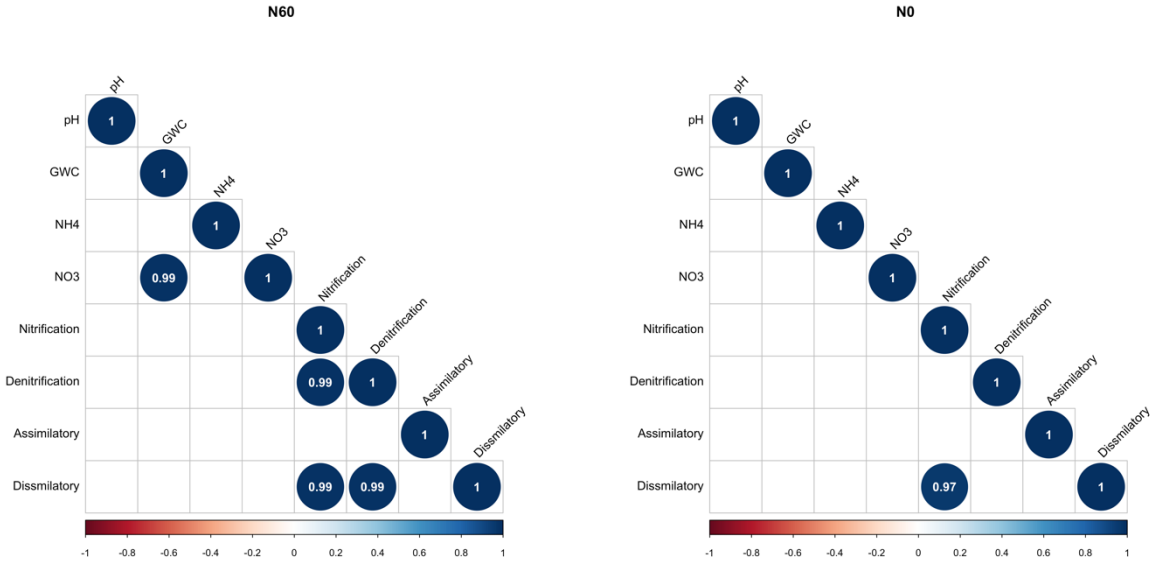


Figure 5.5 Pearson's correlation coefficients among soil properties and genes involved in nitrification, denitrification, assimilatory nitrate reduction, assimilatory nitrate reduction.

False discovery rate was controlled using Benjamini and Hochberg method (1995). The number within cycle represent the correlation coefficient (p-adjusted < 0.05). Color scale represented the value of correlation coefficients.

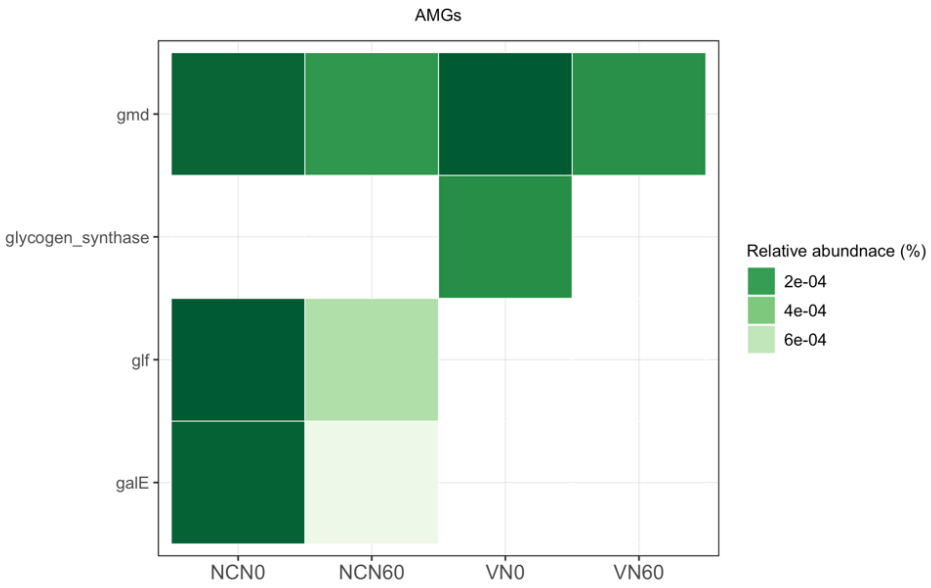


Figure 5.6 Heatmaps for abundances of AMGs related to C decomposition across cover cropping and fertilization treatments.

The distribution of UDP-glucose 4-epimerase [EC:5.1.3.2](*galE*), UDP-galactopyranose mutase [EC:5.4.99.9](*glf*), glycogen synthase [EC:2.4.1.11](glycogen_synthase), GDP-mannose 4,6-dehydratase [EC:4.2.1.47](*gmd*) across 12 samples. The darker color, the greater relative abundances.

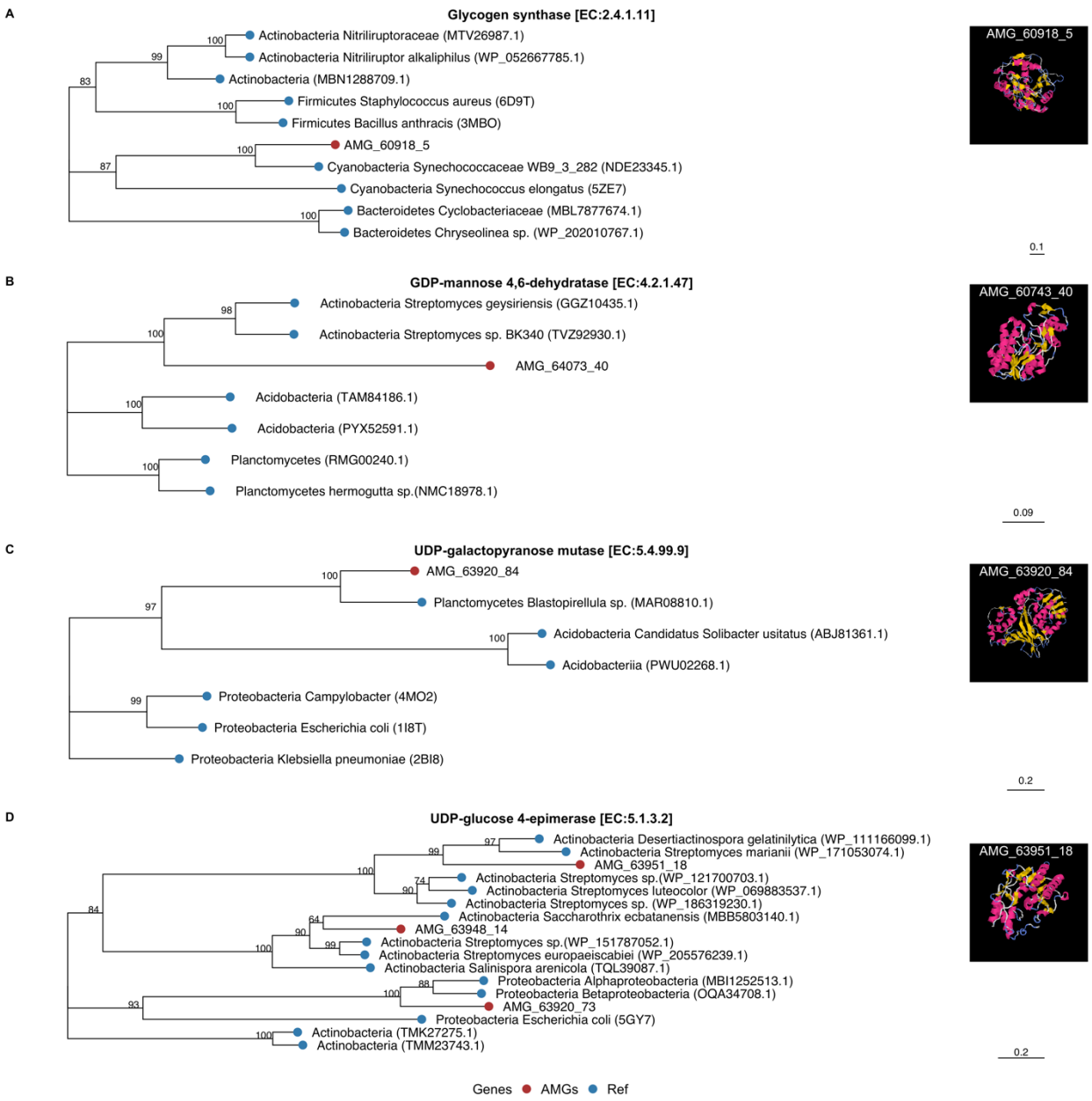


Figure 5.7 Characterization of four auxiliary metabolic genes and microbial genes.

The phylogeny trees were constructed one AMG for each group based on protein sequences via FastTree. Their first 3D structure model predicted by I-TASSER were shown top-right corner of each figure. The tip point colored by blue, and red represent the genes obtained from references

in NCBI and I-TASSER, AMGs from vOTUs in our study, respectively. The scale bar next to the tree suggests the number of substitutions per site.

Chapter 6 Conclusions

This study explores the response of bacterial and viral diversity, and microbial functional genes across inorganic N fertilization, cover cropping, and conventional tillage, and novel viruses and their correlations with microbial hosts. There is evidence that viruses indirectly impact C cycling through viral lysis to influence the quantity of dissolved organic C, viral abundances correlated with bacterial functional taxa with the capability for C decomposition, and viruses can directly involve in C-decomposition via auxiliary metabolic genes. These are significant findings for studying soil viral diversity and their functions in agricultural systems. The results of this study contribute to the study of soil virology and the roles and contribution of viruses in biogeochemical cycling. My major findings in each chapter of this dissertation are summarized as follows.

1. Long-term tillage, fertilization, and cover cropping management practices contribute to the structure of bacterial communities. The organic matter input to soils from different cover cropping treatments and different moisture contents might alter the mechanisms of adsorption or distribution of viruses and differentiate the viral communities. Greater soil water content under vetch treatment increases bacterial and viral abundances. Our results indicate that increases in viral abundance potentially promote release of readily available carbon possibly originating from lysed host cells, resulting in increased EOC. This bioavailable C could then be taken up and metabolized by surrounding bacteria, thus reshaping the bacterial communities.

2. Bacteria communities were influenced by long-term agricultural management practices and seasonal climate variation. N fertilization and tillage had a stronger and lasting effect

on the structure of bacterial communities relative to the tillage and cover cropping management. Alpha diversity of the bacterial community was only influenced by tillage. Cover cropping had a short-term effect on structure of bacterial communities and occurred after burning down of cover crops. Bacterial taxa known to be associated with N fixation, nitrification, and denitrification were directly and strongly influenced by tillage, vetch cover, and air temperature, and soil moisture content.

3. The 260 vOTUs including 232 novel viruses and 28 previously reported were identified from twelve bulk soil metagenomes. Multiple vOTUs at genus or higher level can be potentially linked to the microbial genus level or higher level within same phyla. Microbial host populations were identified that are the key C and N cycling taxa including *Acidobacteriota*, *Proteobacteria*, *Verrucomicrobiota*, *Firmicutes*, and ammonia oxidizing archaea (AOA) *Nitrososphaeria*. Long-term inorganic N fertilization has a greater effect on influencing viral alpha-diversity, community structure, and positively correlation between viruses and their potential hosts than cover cropping.

4. Inorganic N fertilization might promote relatively labile C substrate decomposition by increasing gene abundances in decomposing hemicellulose and cellulose. Genes coding for pectin and chitin decomposition increased in abundance under no cover cropping treatment compared with vetch covered soil. The results indicate that N fertilization and cover cropping have a positive effect on soil C storage by providing N and readily C resources. Restrictions on N and C input may redistribute into the labile soil organic C pool and dampen soil recalcitrant C pool inputs.

Inorganic N fertilization increased nitrification, denitrification, dissimilatory nitrate reduction relative to cover cropping. Significant positive correlations between dissimilatory nitrate reduction, denitrification were found under both N fertilizer addition and no fertilizer treated soil, suggesting that microbes developed the strategy to conserve N to maintain N nutrient levels via dissimilatory nitrate reduction. The structure of microbial C degradation and N-cycling genes across samples were strongly influenced by inorganic N fertilization compared to cover cropping in over 40-year continuous cotton plantation system.

In addition, AMGs identified from viruses may play important roles in microbial energy production and storage, receptor recognition, etc. Those genes may originate from *Cyanobacteria*, *Planctomycetes*, *Proteobacteria*, and *Actinobacteria* phyla. This study provides crucial information on how soil microbes C degradation and N-cycling genes response field managements and reveals the importance of soil viruses on influencing microbial C metabolism through AMGs.

We found that long-term conventional tillage, fertilization, and cover cropping can shift the diversity of bacteria communities, and these management systems tend to enhance the connection between microbial functional taxa involved in N cycling. Functional genes related C substrate verify that fertilization and cover cropping have the potential to improve soil C storage and promote N-cycling.

The soil viral community, their correlation with microbial hosts, and contribution to soil C and N cycling are remain at the early stage. Our findings provide insight into connection novel soil viruses and host, and evidence for capability of viruses on influencing C-degradation, which revealed the key roles of viruses in soil system. The RAPD-PCR approach used in the first chapter of this study to assess differences in viral genetic diversity suffers from low resolution and lack of taxonomic information to explore the correlation between virus and bacteria communities at genetic level. Utilizing the bulk-soil metagenome is a more effective approach for detecting both viruses and their host, thus viromics as a complement on studying viral ecology is suggested in future study.

VITA

Ning Duan was born in Xinzhou, Shanxi Province, China on December 28th,1991 and obtain her bachelor's degree from Soochow University in 2014. After another 3-year study for her microbiological master's degree in Nanjing University, China, she spent 4 years in University of Tennessee, Knoxville, U.S. to complete her doctoral research about the response of microbial and viral community to tillage, cover cropping, and fertilization management using bioinformatic techniques. All her research from 2010 to 2021 were focused on environmental microbiology, including fungi, bacteria, archaea, and viruses. Ning Duan completed her dissertation defense in 2021.