To the Graduate Council:

I am submitting herewith a thesis written by Clara Christina Pregitzer entitled "Plant genotype and environment interact to influence soil carbon and nitrogen dynamics." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

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Plant genotype and environment interact to influence soil carbon and nitrogen dynamics.

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Master of Science

Degree

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Abstract

Abiotic and biotic variation has been shown to be important in regulating nutrient cycling and belowground communities in natural systems. However, genetic variation in dominant plants as a driver of rates of nutrient cycling is still poorly understood and few studies have looked at genotype interactions across multiple environments. Using *Populus angustifolia* and a common garden approach, we hypothesized that all three factors: tree genetic variation, environmental conditions and genetic by environment (G x E) interactions would affect soil carbon (C) storage and nitrogen (N) cycling. Replicated copies of five different reciprocally planted *Populus* genotypes were studied in three separate 18-21 year old common gardens at different elevations (1300m, 1384m and 1587m) in northern Utah, to measure the genotype and environmental effects on pools of soil C and N as well as rates of soil net N nitrification and net mineralization. Our results indicate that genotypes influence pools of soil C, total N and C:N, but genotype did not influence net rates of nitrogen mineralization. Environmental variation significantly influenced pools of soil C, total N, soil C:N and rates of net nitrification and net N mineralization. As predicted, G x E interactions significantly influenced both pools and processes of soil C and N cycling. Overall, we found that genetic variation in plant traits (tree diameter and leaf/root chemistry) as well as soil texture across gardens were significant predictors of soil C and N pools and fluxes across seasons. These data help us understand the relative role of genotypic variation on above- and belowground interactions in different environments and the consequences of these interactions on ecosystem processes. The results from this study show that across an environmental gradient *Populus angustifolia* genotypes can influence nitrogen mineralization through feedbacks between environmental variation, tree phenotype and soils.

TABLE OF CONTENTS

Introduction 1
Populus angustifolia genotypes 4
Soil analyses 5
Statistical Analyses 7
Results 7
Populus angustifolia phenotypes 7
Soil Analyses 8
Soil Nitrogen Cycling 9
Mechanisms 10
Discussion 11
Conclusions & Implications 15
Literature Cited 17
Appendix 25
VITA 35

LIST OF TABLES

Table Page

Table 1 1

Table 2 27

Table 3 28

Table 4 29

Table 5 30

LIST OF FIGURES

Figures Page

Figure 1 32

Figure 2 33

Figure 3 34

Introduction

While it has long been recognized that biodiversity in forested ecosystems can maintain ecosystem processes such as aboveground productivity, decomposition, nutrient cycling, and carbon (C) sequestration, only recently have studies shown that intraspecific genetic diversity can be just as important to the maintenance of these processes (Weis & Abrahamson 1986, Madritch & Hunter 2002, Madritch et al. 2003, Johnson & Agrawal 2004, Schweitzer et al. 2004, Fischer et al. 2005, 2006, Lowjeski et al. 2009). Multiple common garden studies clearly demonstrate that plant genetic variation can regulate species interactions, community structure and ecosystem processes. For example, Madritch & Hunter (2002) found that variation in tissue chemistry across litter phenotypes (*Quercus laevis*) influenced leaf litter decomposition rates and ultimately C and N fluxes. Similarly, Schweitzer et al. (2004) found (both in the field and in common gardens) that variation in leaf condensed tannins (CT) across stands that vary in *Populus* overstory composition can account for significant variation in annual rates of net N mineralization and net nitrification (explaining 60-66% of the variation in net N mineralization). While emerging studies are demonstrating the importance of the biotic inputs of dominant plant species and even genotypes to the maintenance of ecosystem function, these processes are complex, and plants traits are strongly influenced by abiotic environmental factors (Pastor et al. 1984, Finzi et al. 1998, Franzluebbers et al. 1999). Therefore understanding the relative role of genetic vs. environmental variation in regulating ecosystem processes fills an important gap in understanding ecosystem function over time and across landscapes (Madritch et al. 2009, Fischer et al. 2010).

Because ecosystem processes, such as soil C and N dynamics, can be directly linked to their associated plant and soil phenotypes, across genotypes and environments one would expect to see: 1) phenotypic plasticity (G x E) in nutrient dynamics (sensu Conner and Hartl 2004, Bailey et al. 2009); and 2) the strength of plant genetic factors on ecosystem processes to decline relative to the plant phenotype with increasing environmental variation (Johnson and Agrawal 2005, Bangert et al. 2006, Bailey et al. 2009). For example, in a leaf litter decomposition experiment, Madritch et al. (2006) found that under two different nutrient treatments (environments) *Populus tremuloides* genotypes significantly differed in rates of leaf litter decomposition. Overall, nutrient treatment had the strongest effect and genotypes responded differently to the nutrient treatments showing significant G x E interactions. Using a hybridizing system, Fischer et al. (2010) found that there were strong genetic controls on soil N availability below *Populus* crosstypes and this was consistent across river drainages (no G x E effect), however soil phosphorus (P) concentrations were more strongly regulated by environment (i.e., river drainage) and a significant G x E effect was found indicating that control of P cycling is strongly influenced by environment. Clearly the interplay of genetic and environmental variation can influence ecosystem level processes yet the strength of these relationships across systems and direct measures of an ecosystem level response to genetic variation across environments are just beginning to be quantified and understood.

. Using G x E studies we can trace how genetic variation responds to environmental pressures and feeds back to influence variation in phenotypes and ecosystems. For example, Madritch et al. (2009) found that *Populus* genotypes varied across an environmental gradient in leaf chemistry which was tightly correlated with belowground C and N cycling. They found that soil C and N traits as well as soil microbial activity was related to genetic distance among

clones, showing that environmental pressures select for shifts in the genetic stock of forest stands over time leading to distinctly different rates of nutrient processing. Here we utilize three 19-21 year-old common gardens (with common genotypes planted in each) across a 300m elevational gradient to test the role of *Populus angustifolia* genotypes on soil C and N pools and annual rates of net N mineralization and net nitrification. Using a common garden approach with common *Populus angustifolia* genotypes reciprocally planted across elevations we can test the strength of genotype, environment and the extent of these interactions (G x E). Based on previous studies we expect that 1) genotypes vary in phenotypic traits which will influence C and N pools and nitrogen cycling 2) climatic variation found in the three common gardens spanning ~300 m in elevation, will have strong influences on both plant traits and subsequent soil processes, such that a G x E response will be found whereby genotypes will respond differently (above- and below ground traits) to each garden relative to one another resulting in different inputs to soil nutrient pools and process rates.

Materials and Methods

Common garden environments

To determine the influence of plant genotype and environment on plant-soil linkages we utilized three common gardens planted at different locations along the Weber River in Utah, USA. For this study, we used five replicated *Populus angustifolia* genotypes, which were randomly and reciprocally planted in each garden (3-5 clonal replicates of each genotype per garden, n=68 total trees). The Ogden Nature Center garden in Ogden, UT (41.26N, 112.11W) (~1300m), was planted in 1991, the North Pit garden, Uintah, UT (41.49N, 111.56W) (~1384m) was planted in 1989, and Taggart garden, Taggart, UT (41.57N, 111.19W) (~1594m) was planted in 1988 (Table 1). Each garden is within 500 m of the Weber River and all three

common gardens are separated by >20 km. All common gardens are composed of trees that were propagated from local field trees of known genotypes, previously determined by restriction fragment length polymorphism (RFLP; Keim et al. 1989, Martinsen et al. 2001). Mean, minimum, and maximum annual precipitation and temperature patterns for each garden were compiled using weather station data (all stations < 3km from garden sites) from 1988-2005 (University of Utah Climate; http://climate.usurf.usu.edu/). The gardens differ in mean annual precipitation by 150mm (448-600mm) and mean annual temperature by 3˚C (7.7-10.7˚C). All soils in these forests are alluvial, sandy-loams characterized as mesic Oxyaquic Haploxeroll (low elevation, Nature Center), mesic Typic Haploxerolls (intermediate elevation, North Pit), and frigid Fluventic Haploxeroll (high elevation, Taggart) (Table 1).

Populus angustifolia genotypes

To determine variation in tree phenotype and biotic inputs between genotypes across the common gardens we assessed a suite of plant growth and chemical traits collected from each tree across all common gardens. These differences allowed us to determine the influence of genotype, environment and G x E interactions on plant phenotype (tissue quality and an estimate of productivity) and to compare to soil C and N pools and process rates among genotypes. Differences in tree diameter (DBH) growth among *P. angustifolia* genotypes were measured once during June of 2009. These values were used in allometric equations for the same tree species and sites to determine the aboveground woody biomass of each individual tree (based on allometric equations in Lowjeski et al. 2009); we will present the data however as DBH. To determine differences in foliar nutrient inputs and tissue quality among *P. angustifolia* genotypes across each common garden, we measured concentrations of lignin and condensed tannins on

plant leaf and root tissues collected from each genotype. To collect leaf litter, a mesh bag was tied around one random branch from each tree before leaf senescence and the litter was then collected in early December 2008. To quantify variation in root tissue quality among genotypes and across gardens, we collected ~10g of fine roots (<2mm) from beneath each tree during June 2009 (from 0-15cm). The roots were gently washed in deionized water to remove soil before air-drying. Both leaf litter and roots were air-dried and ground to 20 mesh on a Wiley Mill. To quantify lignin concentrations in the ground leaf litter and root tissues, we used the acid-fiber detergent method using an Ankom 200 fiber analyzer (Ankom Technology, Macedon NY); *Quercus rubrum* leaf litter was used as a standard. Condensed tannins were extracted from the ground leaf litter and root tissues with 70% acetone + 10m M ascorbic acid and then assayed using the butanol-HCl method, using purified condensed tannin from *P. angustifolia* as a standard (Hagerman and Butler1989). All tissue data are presented on a percent air-dry mass basis.

Soil analyses

We quantified pools of soil organic C and total N as well as annual rates of net N mineralization and net nitrification in soils beneath all genotypes in all gardens to determine the relative role of plant genotype, environment and G x E interactions. Total organic C and total N were determined on air-dried soils collected from beneath each tree (June 2008) using a Thermo CHN analyzer (Thermo Electron, Milan Italy). Field incubations were conducted beneath each replicate genotype to assess soil net N mineralization rates between genotypes and across gardens from June 2008-June 2009 with four sequential incubations throughout the year, at the seasonal boundaries. The summer and winter incubations lasted approximately 3.5 months while

the spring and fall incubations lasted approximately 2.5 months. On the east and west side of each tree in the common gardens, two polycarbonate soil cores (4.8 x 15cm) were inserted <0.25m from the base of each tree trunk into the soil to a depth of 15cm (0-15cm). One of the soil cores was removed immediately and taken back to the laboratory to determine initial soil inorganic-N pools, the other soil core was left in the soil to incubate over the course of the season when relative soil moisture and temperature was similar to bulk soil averages. Upon removal from the field, soils were transported immediately to the laboratory on ice and were kept cool (~4°C) until processing, within 36 h. Soils were sieved (<4 mm) to remove any coarse fragments and roots and one 20 ml sub-sample was placed in a specimen cup, and immediately extracted with 100 ml of 2M KCL. Each extract was shaken for 1 h, gravity-filtered with Whatman filter paper no.1 (first leached with deionized water and 2M KCL) and stored in at 0º C until analyzed for extractable ammonium ($NH4^+$) and nitrate ($NO3^-$) (Lachat AE auto-analyzer; Lachat Industries, Inc., Loveland, CO, USA). Annual rates of N mineralization ($NH_4^+ + NO_3^-$) and net nitrification were calculated by summing the seasonal rates. The incubated soils were collected at the end of each season and processed in the same manner (Raison et al. 1987). A sub-sample from each soil sample from each collection date was oven dried (48 h at 105 °C) to determine soil gravimetric water content. All final soil N data are presented on an oven-dry mass basis.

Additionally, two soil samples taken from beneath each tree during the June 2008 collections were pooled, air-dried and used to determine soil pH using the 0.1 M CaCl method (Hendershot et al. 1993). Soil texture (i.e. particle size) was determined for each genotype (replicate samples pooled with common gardens) using the hydrometer method (Gee & Bauder 1986).

Statistical Analyses

Net N mineralization data were analyzed using a mixed model, repeated measures MANOVA with time as a factor on log +1 transformed genotype data across all seasons to determine the relative influence of genotype, common garden environment, and G x E interactions (Wilkes Lambda was used for all repeated measure tests). Seasonal rates of net N mineralization and net nitrification were analyzed using a generalized linear mixed model, two-way ANOVA. All other abiotic and biotic data collected from the common genotypes across gardens was analyzed using a mixed model, two-way ANOVA on normalized genotype means to determine the relative influence of genotype, common garden and G x E interactions. To determine the most important mechanisms influencing soil nutrient pools and process rates across gardens and seasons, stepwise linear regression analyses were conducted using all normalized data, *P. angustifolia* traits (plant growth and tissue quality) were correlated with soil factors, as well as with net N mineralization and nitrification rates (JMP 8.0 was used for all analyses, SAS company).

Results

Populus angustifolia phenotypes

Consistent with our hypothesis, we found plant growth and plant tissue traits varied by genotype, common garden and in some cases a G x E interaction, all of which could differentially impact soil processes. We found significant differences in tree diameter (and woody biomass) by genotype, environment and a significant G x E interaction, indicating the role of both plant genotype and the climatic differences across the three common gardens (Table

2) on tree growth. Trees at the Nature Center (the lowest elevation) common garden, overall, had the greatest diameter being 9.5-11.5cm greater on average than the two higher elevation gardens.

The concentrations of both lignin and condensed tannin in plant tissues can slow litter decomposition and nutrient release and we found tissue-specific patterns in these compounds among genotypes and common garden environments, however no G x E interactions (Table 2, 3) were significant. Leaf lignin and leaf CT were significantly different among the genotypes (Table 2, 3) and across the five *P. angustifolia* genotypes leaf lignin differed between 1.3-11.5% and leaf condensed tannin differed between 1.3-5.8% (Table 2). Neither root lignin nor condensed tannin varied significantly by genotype (Table 2, 3). We found that common garden environment had significant effects on tissue quality. The highest % leaf lignin was found at the Nature Center garden (at the lowest elevation), the lowest was found at Taggart (at the highest elevation); North Pit (intermediate elevation) was not significantly different than the Nature Center common garden (Table 2). We found no significant differences for root lignin ($p=0.059$, Table 3) across common garden environments. We found leaf CT to vary significantly across common garden environments (Tables 2, 3) where leaf CT was significantly greater at North Pit (intermediate elevation); however, there was no significant difference between Nature Center and Taggart (Tables 2, 3). Root tannins also varied significantly among common gardens (Tables 2, 3). There were no significant G x E interactions for leaf or root lignin or concentrations of condensed tannins (Tables 2, 3).

Soil Analyses

Soil factors (pH and texture) that are known to influence C and N pools and process rates did not vary significantly by genotype (Table 2, 3), but did vary significantly by common garden

environment as well as demonstrating a significant G x E interaction. Nutrient pools measured as soil organic C (% C), total N (%N) and C:N ratio were found to vary significantly by genotype, common garden environment and sometimes demonstrated a G x E interaction. Soil % C varied between genotypes by 0.18-3.94%, soil % N from 0.01-0.24% and C:N ratio from 0.18-6.50, respectively (Table 2, Figure 1). We found significant differences in soil % C, % N and C:N ratio between the three common gardens (Table 2, 3, Figure 1). The greatest soil % C and % N was found at the Nature Center garden, North Pit had the lowest concentrations and Taggart exhibited intermediate values (Table 2, Figure 1). The C:N ratio was highest at the Taggart, intermediate at Nature Center (but not significantly different from Taggart), and lowest at North Pit (Figure 1, Table 2). Significant G x E interactions were found for soil % C, C:N ratio, and soil pH but not %N (Table 3) indicating soil nutrient pools are plant-mediated and sensitive to variation among genotypes whereby the rank order of these values under genotypes change across environments.

Soil Nitrogen Cycling

Overall, annual rates of net N mineralization and net nitrification did not vary significantly by genotype ($p=0.23$, Figure 1, Table 4); however they were significantly different across common garden environments, and there were also significant G x E interactions (Table 4, Figure 3). The greatest annual rates of net N mineralization were found at the low elevation garden (Nature Center, 54.0 mg N/kg), and these rates were roughly three times higher than the rates found at the high elevation garden (Taggart, 18.19 mg N/kg). While there were no significant differences in annual rates of net N mineralization and nitrification among genotypes, noticeable differences were evident in that annual net N mineralization was approximately two times greater between genotypes in the same garden (Figure 1).

Seasonal differences are important to take into consideration because seasonal fluctuations in N mineralization will likely follow weather patterns that impact soil microbial activities, tree assimilation of soil nitrogen, and leaf senescence. Seasonally, net N mineralization and net nitrification did not vary by genotype (except for NO_3^- during the winter season, $p=0.04$), but did significantly vary during every season across common garden environments (Figure 3), and during some seasons (spring and summer) a significant G x E interaction was found. Shifts in peak N process rates varied across seasons for each garden. The highest rates of net N mineralization occurred at the Nature Center in the fall (16.32 mg N/kg), the highest rates of net N mineralization at North Pit occurred in the summer (11.26 mg N/kg) and at Taggart the highest rates of net N mineralization during the spring (6.51 mg N/kg) (Figure 2), likely reflecting the effects of climatic differences on organic matter decomposition rates across time. Overall, environment had the most significant effect on N cycling where the low elevation garden (Nature Center) had the greatest rates of net N mineralization seasonally as well as annually. Even during its lowest season (winter, 7.31 mg N/kg) trees at the Nature Center garden still mineralized more N than the highest elevation garden during its peak season (Taggart gardens greatest N min was during the spring, 6.51 mg N/kg; Figure 2). Annual rates of net N mineralization and net nitrification exhibited significant genotype by common garden (G x E) interactions (Table 4), while soil processes beneath genotypes vary relative to one another in different common garden environments. When analyzed seasonally, significant G x E interactions also occurred (Table 4).

Mechanisms

Linking the patterns we see in plant phenotype and soil C and N pools and N processes rates helps connect above-and below ground processes and by identifying these putative

mechanisms we can begin to understand the importance of genetic differences thus connecting genes to ecosystems. The factors influencing soil nutrient pools (% C and % N) most strongly were tree diameter and soil factors (pH and % sand). Of all the plant and soil factors, tree DBH ($p<0.0001$, $R^2=0.31$) and % sand ($p=0.002$, $R^2=0.13$) explained the most variation in pools of total soil % N. Soil pH ($p=0.018$, $R^2=0.414$) and tree DBH ($p=0.0003$, $R^2=0.12$) influenced pools of soil % C most strongly. Across all potential plant inputs and soil factors, annual rates of net N mineralization were influenced most strongly by soil pH (<0.0001, $R^2=0.24$;Table 5). Across gardens, annual rates of net N mineralization were influenced differently, where at the low elevation garden (Nature Center) annual N mineralization could be significantly correlated with % leaf lignin and % root lignin, and at the intermediate-elevation garden (North Pit) % leaf lignin and tree DBH were significantly correlated and no plant or soil factors could be significantly correlated with annual rates of N mineralization at the high elevation garden (Taggart; Table 5). Seasonal rates of N mineralization were also influenced differently across gardens where during some seasons multiple factors significantly correlated with N process rates and during other seasons no plant or soil factors were significantly correlated, and these patterns varied for each garden (Table 5).

Discussion

Our results support the hypothesis that interactions between plant genotype and environmental variation impact plant-soil linkages, through genotype-specific responses in plant phenotypes, to affect soil C and N pools and annual rates of net N mineralization and nitrification. Overall we found C and N pools and N mineralization and nitrification rates to be most strongly regulated by the environmental differences across common gardens. We also

found that the genetic effects on belowground processes can be regulated by the environment (G x E interactions) as shown by five common plant genotypes interacting with three common garden environments resulting in rank order changes among the genotypes in plant phenotypic traits and rates of net N mineralization and nitrification.

For ~20 years these five genotypes have been influenced by their respective common garden environments and over time strong effects of both genotype and environment (G x E in some cases) on plant productivity and leaf litter chemical traits were found (Table 3). Strong genetic and environmental effects on the pools of organic C and total N (and C:N ratio) in the soil, and G x E interactions for soil organic C and C:N were found suggesting possible links from the plant phenotype to soil C and N pools. This result parallels other studies where variation in above-below ground tissue quality and productivity among species or functional groups can cause shifts in C and N pools and fluxes (Tilman et al. 1997, Finzi 1998, Ehrenfeld 2003). Therefore, we attribute our strong G x E effects on annual rates of net N mineralization and net nitrification to variation in plant phenotype from genotype-specific responses to the environment. These results overall show that the environment has strong effects on plant phenotypes and that responses (i.e., in plant growth and litter chemical traits) can be correlated with organic matter inputs to soils that can influence belowground C and N pools and N process rates. These data further suggest that genotypes may strongly regulate total C and N pools (even across environments) but that rates of N processing are more phenotypically plastic, as these rates are mediated by soil microbial activities that demonstrate high variability in process rates across substrates and environments.

We found strong genetic effects for plant phenotypic traits as well as pools of soil C and N, however we found weak overall genotype effects for annual rates of net N mineralization and nitrification across gardens (i.e., G x E effects only; Table 4). As it has been shown that genetic diversity can shape phenotypes (Lindroth et al. 2002, Rehill et al. 2006), communities (Wimp et al. 2004, Genung et al. 2009) and ecosystem processes (Whitham et al. 2003, Schweitzer et al. 2004, Hughes et al. 2008) just as much as species diversity (Schweitzer et al. 2005; Crutsinger et al. 2006; Johnson et al. 2006) it is important to understand the relative influence of environmental factors as they may mediate biotic or genetic effects (Madritch et al. 2006). In a recent meta-analysis Bailey et al. (2009) showed that the strength of plant genetic effects decrease from the phenotype to the community to ecosystem processes and that below-ground responses are generally weaker than above-ground responses. Our results support this conclusion in that significant environmental and G x E effects were found on annual rates of net N mineralization and net nitrification, suggesting that environmental variation was a stronger driver than genotypic variation. Yet genotype was still important (as shown through significant G x E effects) as the environment had differential impacts on plant genotypes that influenced the strength of plant-soil linkages.

As predicted, the environment had strong effects on both plant phenotypes and plant-soil linkages that influence associated soil processes. Moreover these data suggest that some genotypes were more plastic than others, indicating that there is plasticity in the ability of plants to regulate their soil environment through plant soil feedbacks. As each genotype responds to the environmental pressures (i.e. cooler or warmer temperatures) phenotypic variation becomes evident, where increased productivity (found in trees at the low elevation garden), or higher concentrations of leaf lignin/condensed tannins then caused variation in biotic inputs to the soils

directly beneath each tree. These plant-soil feedbacks are regulated by soil microbial communities which ultimately release nutrients for plant assimilation, with fitness consequences. For example, in this same system it was found that a positive plant-soil feedback occurs where *P. angustifolia* genetic families had fitness advantages (twice as likely to survive, grew 24% taller, had 27% more leaves, and 29% greater above-ground biomass) when grown in soil collected from beneath other *P. angustifolia* trees versus soils collected from other *Populus* species and hybrids in the same river drainage (Pregitzer et al. 2010). Because we saw significant responses in tree phenotypes (genetic, environment, G x E, Table 3) and in soil N process rates (environment and G x E), results from this study show the consequences of genetic variation in multiple environments and that plant genes can regulate plant soil feedbacks through their response to the environment.

While links between plants and soils are apparent, the mechanisms contributing to the variation we see in plant phenotype, soil phenotype and soil processes are complex. Because this study utilized 20 year old common gardens in natural field settings it is difficult to disentangle the environmental variables which are responsible for these strong effects on plant traits, soil factors and C and N pools. Some of the variation we find could simply be an artifact of the site quality (i.e. soil texture). However, differences in temperature and precipitation are apparent at each common garden (Table 1) which could drive some of the patterns we see in tree productivity, leaf litter quality and rates of net N mineralization. For example, a pulse of net N mineralization occurs during different seasons at the three common garden environments indicating that environmental variation can strongly regulate the timing of these cycles (Figure 3). Correlations between net N mineralization and plant and soil traits also show shifts in the timing and significance of biotic inputs across common garden environments seasonally and

annually (Table 5). When seasonal net N mineralization (Figure 3) patterns are matched with plant and soil traits (Table 5), the relative influence of biotic inputs varies across gardens. For example, at the intermediate garden (North Pit) a pulse of N mineralization occurs during the summer (Figure 3) when plant and soil traits can account for 43% of the variation in N mineralization, while at the high elevation garden (Taggart) no significant plant/soil traits are attributed to the pulse of N mineralization during the spring (Table 5). The dynamic properties of ecosystems are clear and our results show the sensitive nature of belowground systems to environmental variation as well as fine scale changes in biotic inputs of organic matter abundance and quality.

Conclusions & Implications.

Many studies have shown strong patterns that the environment (Powers 1990, Garten & Van Miegroet 1994, Garten et al. 1994) and biotic inputs (Schweitzer et al., 2004, 2005, Tilman et al. 1997, Zak et al. 2003, Ehernfeld 2003) can regulate ecosystem function, however few have looked at their interaction (but see Madritch et al. 2006). Understanding the effects of plant genotype (intraspecific variation) on ecosystem processes (e.g., the extended phenotype of plant genotype) with a genetically diverse foundation species, such as *Populus angustifolia* places the study of ecosystem ecology into an evolutionary framework (Whitham et al. 2003, 2006). As selection acts across environmental gradients on this species we can expect to see shifts in the genetic structure (i.e. fitness advantages) or tree phenotypes (i.e. plasticity) of these forest stands which will have lasting effects on the associated communities and ecosystems. Finding a G x E response advocate that genotypic responses to environmental selection pressures vary and because we find this response at the ecosystem level (i.e. N mineralization) it suggests feedbacks

between plants and soils are genetically mediated. Plant-soil feedbacks are just beginning to be placed in an evolutionary perspective (Pregitzer et al. 2010) and show that soil legacy effects due to genetically mediated plant-soil feedbacks can promote the success of some genotypes over others leading to the creation of spatial forest mosaics across landscapes (Thompson et al. 2005, Madritch et al. 2009). These mosaics are evident in our study with phenotypic differences among the same five genotypes planted in different environments which have led to significant differences in plant-soil relationships. As we make predictions for future ecosystems, G x E studies may help us understand the factors influencing ecosystem goods and services (i.e. C and N cycling, water cycling, and productivity), the plasticity of genotypes within a species, and the potential fitness and evolutionary consequences plant-soil linkages in forest stands across environments and landscapes. With changes in the global climate as higher elevation trees begin to experience temperatures outside their natural range of variability, potential shifts in net N mineralization and nitrification could occur, and based on our results we could predict two- to fivefold shifts in net N mineralization rates given an increase in 3°C. In conclusion, we found that environmental variation strongly controls tree phenotypes, soil C and N pools and N cycling rates. While plant genes also strongly influence tree phenotypic variation and soil C and N pools they interact with the environment to regulate rates of net N mineralization and nitrification.

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Appendix

Table 1

Table 1. Characteristics of three large-scale common gardens that were planted between 1988 and 1991 with replicate copies of trees of known genotype.

	Nature Center Garden	North Pit Garden	Taggart Garden
Elevation (m)	1300	1384	1587
Mean Precip.[1] (mm/y)	544	951	835
Mean Temp[1] (ºC)	10.7	9.7	7.7
Soil Type	Oxyaquic Haploxerolls	Typic Haploxerolls	Fluventic Haploxerolls

1

Table 2

Table 2. Mean soil characteristics and leaf traits collected from five *P. angustifolia* genotypes in three common gardens planted in northern Utah, USA.

Garden Genotype*	pH	% sand/silt/clay	% C	% N	C:N	% CT	% RCT	% Leaf Lignin	% Root Lignin	DBH(cm)	Annual N Mineralization (mg N/kg)
Nature Center †	**7.35^{c}**	**49/39/12^{b}**	**5.28^{a}**	**0.35^{a}**	**15.17^{a}**	**2.97^{b}**	**0.84^{b}**	**24.64^{a}**	**31.75^{a}**	**26.08^{a}**	**51.08^{a}**
10	7.42	44/45/12	5.47	0.34	16.13	3.07	0.90	26.86	34.51	27.28	52.59
1000	7.20	73/16/10	5.85	0.38	14.99	5.21	0.82	26.14	34.00	32.50	28.65
1008	7.42	36/51/13	5.67	0.34	16.62	1.77	0.78	23.05	30.43	25.14	59.26
1019	7.20	49/41/10	4.03	0.31	12.68	3.23	1.07	24.95	29.71	9.75	49.38
1023	7.35	43/44/13	4.96	0.37	13.76	3.14	0.78	23.52	30.09	31.02	65.53
North Pit†	**6.96^{b}**	**59/31/10^{a}**	**1.91^{c}**	**0.17^{c}**	**11.37^{b}**	**5.41^{a}**	**1.17^{a}**	**23.41^{a}**	**33.66^{a}**	**14.96^{b}**	**27.19^{b}**
10	6.88	64/26/10	1.63	0.16	10.34	6.15	1.38	28.20	35.24	13.38	20.13
1000	6.97	38/49/13	1.98	0.18	10.99	5.16	0.86	26.18	32.85	14.02	28.21
1008	6.88	36/51/13	1.71	0.14	12.53	4.82	1.10	21.99	32.46	13.60	25.88
1019	7.11	49/41/10	1.99	0.19	10.33	6.01	1.24	22.20	32.65	17.12	29.95
1023	6.97	82/10/8	2.28	0.18	12.65	4.97	1.45	21.96	35.14	16.84	31.80
Taggart†	**7.57^{a}**	**33/49/17^{b}**	**3.83^{b}**	**0.25^{b}**	**15.58^{a}**	**3.51^{b}**	**0.93^{b}**	**18.96^{b}**	**32.38^{a}**	**19.64^{b}**	**18.22^{b}**
10	7.58	34/49/16	3.97	0.26	15.27	7.46	0.87	22.34	33.14	18.36	17.17
1000	7.52	31/51/18	3.83	0.26	14.93	2.85	0.85	19.21	32.66	17.46	15.19
1008	7.57	20/61/19	3.74	0.22	16.80	1.95	1.10	16.70	32.11	13.25	15.79
1019	7.56	52/32/14	3.76	0.26	14.67	2.18	0.99	18.13	32.83	24.83	13.82
1023	7.63	29/52/19	3.86	0.24	16.47	4.26	0.89	19.19	31.20	25.18	29.00

* Mean values for garden (n =25 trees).

† Lowercase letters, for each response variable, indicate significance differences across gardens (at the α=0.05 level).

Table 3

Table 3. Two way ANOVA (GLM) results showing p values for soil and plant traits between genotypes (G), across common garden environments (E) and G x E interactions.

	Soil Traits					Plant Traits				
	Soil pH	**% sand**	**% C**	**% N**	**C:N**	**% LCT**	**% RCT**	**% leaf lignin**	**% root lignin**	**DBH(cm)**
Genotype	0.551	<0.0001	<0.046	0.0148	<0.0001	0.027	0.084	<0.0001	0.728	0.033
Environment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.002	<0.0001	<0.0001	0.059	< 0.0001
G by E	0.036	<0.0001	<0.0001	0.297	0.012	0.114	0.139	0.476	0.794	0.0084

Table 4

Table 4. Repeated measures results (MANOVA) showing annual soil nitrogen cycling rates under *P. angustifolia* genotypes grown in three common gardens in northern Utah.

	Net Nitrogen Mineralized		**Nitrification ($NO3^-$)**		**Ammonification ($NH4^+$)**		**Net N mineralized/Total organic N**	
	F	p	F	p	F	p	F	p
Genotype	1.26	0.23	0.83	0.61	1.56	0.09	0.99	0.45
Garden	**8.64**	**<0.0001**	**7.56**	**<0.0001**	**4.90**	**<0.0001**	**12.66**	**<0.0001**
G X E[§]	**1.83**	**0.01**	**1.90**	**0.0007**	1.22	0.21	**1.61**	**0.03**

Table 5

Table 5. Stepwise results indicating significant soil characteristics and leaf traits contributing to rates of soil net nitrogen (N) mineralization (mg N/kg) collected from five *P. angustifolia* genotypes in three common gardens in northern Utah, USA seasonally from 2008-2009.

Season (N cycled Mg N/kg)	Nature Center				North Pit				Taggart			
	Mechanism	p	F	R^2		p	F	R^2		p	F	R^2
Summer	soil C:N	0.0004	14.48	0.25	% leaf lignin	0.006	9.67	0.21	**NS**			
	% root lignin	0.031	6.45	0.07	soil C:N	0.016	7.00	0.10				
					% sand	0.033	5.27	0.12				
Fall	**NS**				% root CT	0.023	6.00	0.22	% leaf lignin	0.0001	30.03	0.40
									% leaf CT	0.011	8.62	0.04
									% root CT	0.019	7.05	0.06
									soil pH	0.013	8.23	0.09
Winter												
	DBH	0.002	13.98	0.34	% root CT	0.004	10.48	0.14	% leaf CT	0.003	10.79	0.29
	% root CT	0.006	10.81	0.26	soil C:N	0.009	8.6	0.19				
Spring												
	soil C:N	0.007	10.04	0.17	**NS**				**NS**			
	% leaf CT	0.012	8.51	0.33								
Annual	% leaf lignin	0.002	16.28	0.26	% leaf lignin	0.02	8.02	0.15	**NS**			
	% root lignin	0.001	20.41	0.25	DBH	0.005	7.12	0.24				

Figure Legends

Figure 1. Average organic soil A) C:N (C), B) % nitrogen (N), C) % carbon, D) annual rates of net N mineralization and E) net nitrification taken and averaged from soil beneath five *P. angustifolia* genotypes reciprocally planted in three common gardens in Northern Utah ($\pm$ one standard error of the mean).

Figure 2. Average annual rates of A) net nitrification (mg N/kg) and B) net nitrogen (N) mineralization for each common garden environment in Northern Utah, taken from incubations beneath five replicated *P. angustifolia* across genotypes ($\pm$ one standard error of the mean).

Figure 3. Seasonal (2008-2009) rates of Nitrogen (N) mineralization (mg N/kg) beneath five *P. angustifolia* genotypes in three common gardens in Northern Utah. The Taggart garden (A) and North Pit garden (B) mineralizes significantly less N than the Nature Center (C) gardens across seasons ($\pm$ one standard error of the mean).

Figure 1

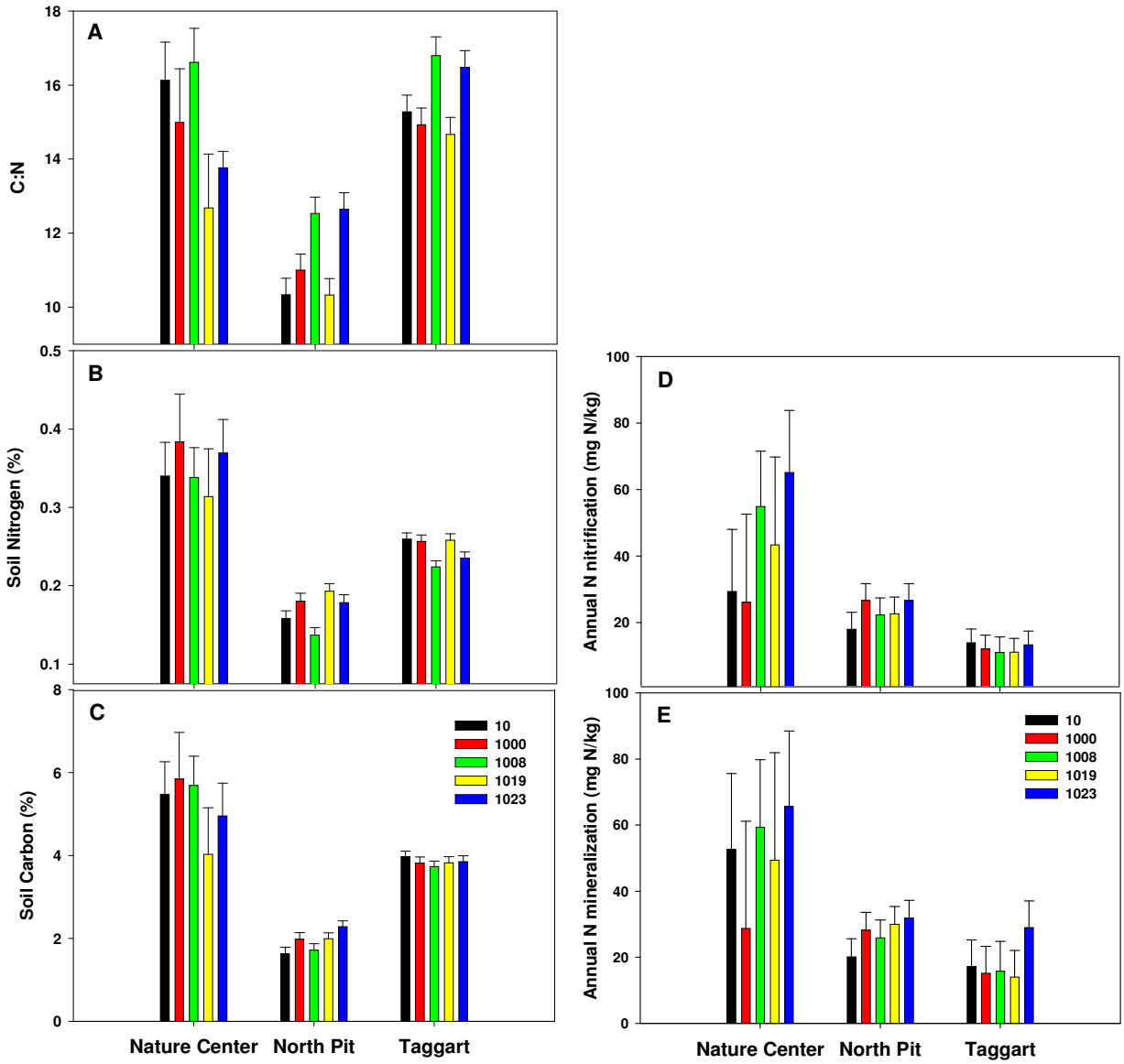

A
C:N
B
Soil Nitrogen (%)
C
Soil Carbon (%)
D
Annual N nitrification (mg N/kg)
E
Annual N mineralization (mg N/kg)
10
1000
1008
1019
1023
Nature Center
North Pit
Taggart

Figure 2

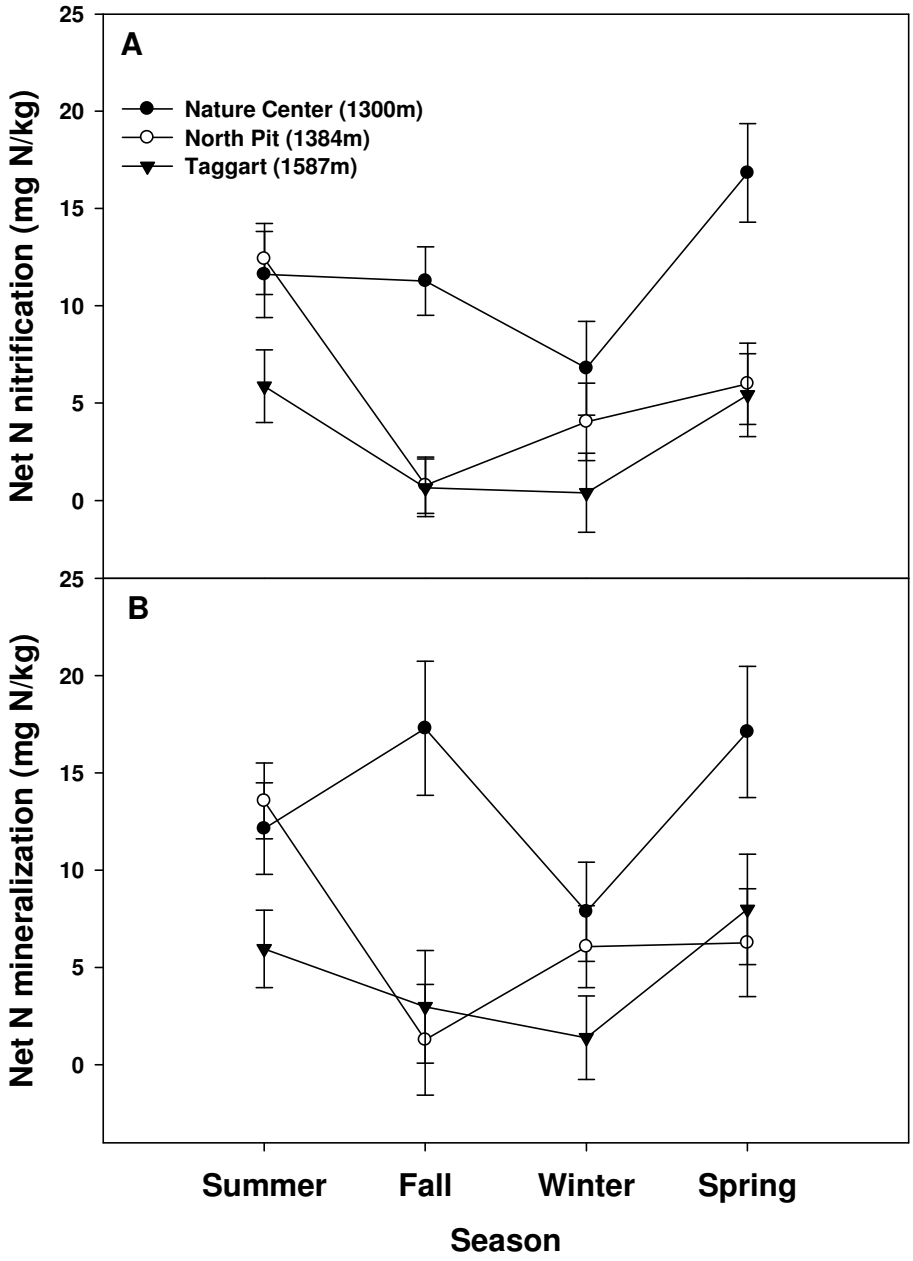
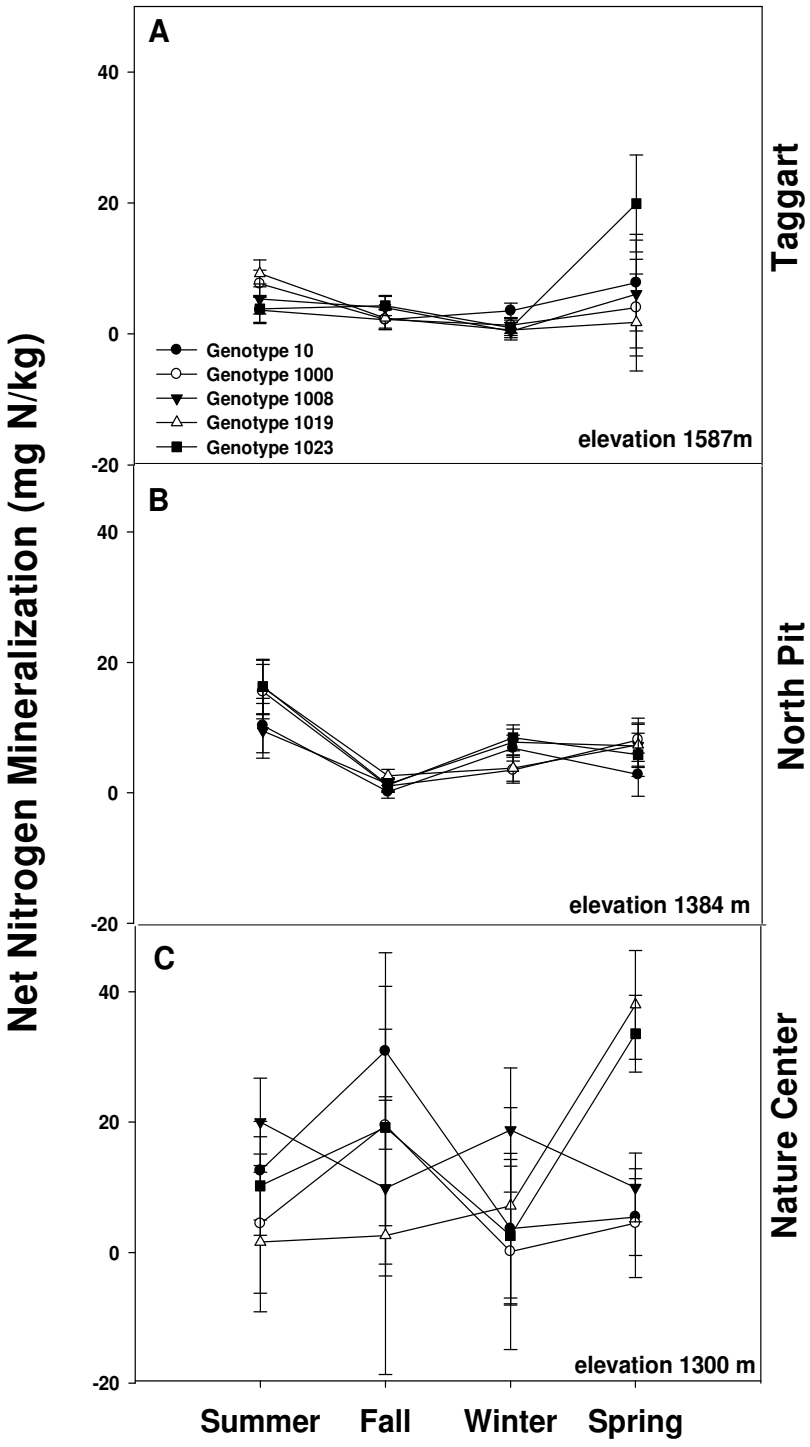

A
B
Nature Center (1300m)
North Pit (1384m)
Taggart (1587m)
Net N nitrification (mg N/kg)
Net N mineralization (mg N/kg)
25
20
15
10
5
0
Summer
Fall
Winter
Spring
Season

Figure 3


A
B
C
40
20
0
-20
Genotype 10
Genotype 1000
Genotype 1008
Genotype 1019
Genotype 1023
elevation 1587m
elevation 1384 m
elevation 1300 m
Taggart
North Pit
Nature Center
Net Nitrogen Mineralization (mg N/kg)
Summer
Fall
Winter
Spring

VITA

Clara was born in Lansing, MI in 1984 and spent the first eight years of her life in Okemos, MI where her family owned and operated a small Christmas tree farm. In 1992 her family moved to Houghton, MI where she attended Houghton Public Schools. She was actively involved in high school athletics as well as the Ecology club which he traveled with to Central America in 2001. She graduated high school in 2002 with honors and immediately following graduation enrolled in the National Outdoor Leadership School where she spent 30 days backpacking through the Wind River Range in Wyoming. In the Fall of 2002 she enrolled at Michigan State University and attended until 2004 when she transferred to Northern Arizona University as a Forestry student. At NAU she worked in the Ecosystem Ecology Lab as well as the Ecological Restoration Institute. As a research assistant with the Ecological Restoration Institute she participated in a wide variety of restoration projects in the southwest focused on restoring healthy forest stand structure, fire patterns and ecosystem processes to this area. As a research assistant with the Ecosystem Ecology lab she initiated an independent project to understand the genetic basis and evolutionary consequences for interactions of plants and soil in the restoration of ecosystems which is now published in *Evolutionary Ecology*. She graduated with a B.S. in Forestry with honors in May 2007 and upon graduation worked in Northern Utah as a research garden manager. At this point she felt she could make a positive contribution to the field of ecology by continuing her education and followed her undergraduate advisors (Jen Schweitzer and Joe Bailey) to the University of Tennessee to work on a M.S. in Ecology. Following graduation she is going to work as a Forest Ecologist in New York City with the Natural Resources Group as part of New York City Parks and Recreation Department.