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Plant diversity and density predict belowground diversity and function in an early successional alpine ecosystem

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Abstract. Despite decades of interest, few studies have provided evidence supporting theoretical expectations for coupled relationships between aboveground and belowground diversity and ecosystem functioning in non-manipulated natural ecosystems. We characterized plant species richness and density, soil bacterial, fungal and eukaryotic species richness and phylogenetic diversity (using 16S, ITS, and 18S gene sequencing), and ecosystem function (levels of soil C and N, and rates of microbial enzyme activities) along a natural gradient in plant richness and density in high-elevation, C-deficient soils to examine the coupling between above- and belowground systems. Overall, we observed a strong positive relationship between aboveground (plant richness and density) and belowground (bacteria, fungi, and non-fungal eukaryotes) richness. In addition to the correlations between plants and soil communities, C and N pools, and rates of enzyme activities increased as plant and soil communities became richer and more diverse. Our results suggest that the theoretically expected positive correlation between above- and belowground communities does exist in natural systems, but may be undetectable in late successional ecosystems due to the buildup of legacy organic matter that results in extremely complex belowground communities. In contrast, microbial communities in early successional systems, such as the system described here, are more directly dependent on contemporary inputs from plants and therefore are strongly correlated with plant diversity and density.

Key words: bacteria; biodiversity; C and N; DNA sequencing; environmental gradient; fungi; microbial community; talus.

Introduction

Terrestrial ecosystems operate with aboveground and belowground subsystems that are inherently linked with each other (Bardgett and Wardle 2010, Bardgett and van der Putten 2014). Within the aboveground subsystem, plants generate organic carbon (primary production) that supports the belowground subsystem (Wardle et al. 2004). In return, within the belowground subsystem, decomposers, mutualists, herbivores and pathogens drive soil processes (e.g., nutrient turnover) that ultimately affect plant growth (Wardle et al. 2004, Bardgett and Wardle 2010). In theory, the links are expected to be positive, namely increasingly diverse and productive plant communities providing more diverse and abundant food resources should support more diverse and abundant belowground communities. In response, more diverse and abundant soil biota regulating decomposition and nutrient dynamics should affect the diversity and productivity of plant communities (Hooper et al. 2000).

Despite this logical framework, evidence for a strong link between the richness of plants and soil groups and ecosystem processes is mixed. Although manipulative experiments that rely on artificially assembled plant or microbial communities (e.g., Wagg et al. 2014, Lange et al. 2015, Weisser et al. 2017) often affirm the expected positive relationship (Tilman et al. 2014, Eisenhauer et al. 2016), observations from naturally assembled communities are less consistent and often deviate from the expected positive relationship. For instance, a study involving a plant richness gradient along a fire-driven retrogressive chronosequence of Swedish boreal forests on 30 islands demonstrated that richness and diversity of belowground microbes and nematodes were not responsive to increasing plant richness, and C storage (as an example of ecosystem functioning) was mainly driven by shifts in plant community composition and declining soil fertility (Wardle et al. 2012). Another study of 25 temperate grasslands around the world representing a wide range of plant diversity and environmental conditions (including C and N pools) provided little support for positive relationships between alpha-level diversity of plants and microbes (bacteria and fungi) (Prober et al. 2015) echoing results of earlier findings (e.g., Porazinska et al. 2003) and leading to a conclusion that in terms of taxonomic richness, plants, soil biota, and ecosystem processes are largely de-coupled (Wardle 2006, 2016). This lack of agreement between experimental and observational studies has been attributed to a wide range of causes including variation of environmental conditions (e.g., Prober et al. 2015), differences in spatio-temporal scales between plants and microbes (Bardgett et al. 2013), and poor equivalence between randomly assembled experimental and non-randomly assembled natural communities (Eisenhauer et al. 2016, Wardle 2016).

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Most studies investigating the link between richness of plants and soil biota and ecosystem function have been conducted in ecosystems with well-established vegetation (i.e., grasslands and forests) (e.g., Wardle et al. 2012, Tedersoo et al. 2014, Prober et al. 2015). In addition, these ecosystems have relatively large and complex pools of organic C (legacy of plants and microbes). We propose that within such ecosystems, plant and soil biota richness relationships and their effects on ecosystem processes may be undetectable because plants and microbes not only can interact directly by using each other's contemporary resources (e.g., labile rhizodeposits used by microbes and simple organic and inorganic products of microbes used by plants), but also less directly by using legacy C (e.g., more recalcitrant compounds) and in effect appear to be de-coupled. In contrast, under soil conditions where legacy C is largely absent (e.g., early successional ecosystems), plant/soil biota interactions should be more direct and more readily detectable. Moreover, an increasing density of plants in pararell with increases in richness could strengthen this coupling.

One ecosystem that lacks substantial legacy C is the high alpine where oligotrophic soils are being colonized by plants as a result of longer growing seasons due to global warming (Pauli et al. 2012, Bueno de Mesquita et al. 2017). The first colonizers in these C-deficient soils are photosynthetic microbes (e.g., cyanobacteria and green algae) which provide carbon and nitrogen that support further colonization by plants and heterotrophic microbes (Schmidt et al. 2008*a*, Freeman et al. 2009). As more plants are added to the community, the diversity of C compounds increases (Schmidt et al. 2008*a*), which should increase the richness of microbes and their biogeochemical outputs. Here we use a natural gradient in the high-alpine zone extending from bare to increasingly vegetated soils to examine the relationship between plant richness, soil biota richness, and ecosystem processes. In this high-alpine ecosystem (3,700–4,000 m.a.s.l.), plant cover has increased at a rate of ~5% per decade over the last four decades in response to climate change (Bueno de Mesquita et al. 2018*a*), but legacy C remains low. In this slowly changing system, we predicted that (1) an increase in plant species richness (and density) would be coupled with an increase in soil biodiversity, and (2) an increase in both plant and soil biota richness would be coupled with an increase in soil C and N pools as well as rates of microbial activity.

Methods

Study site

This study was conducted at sites previously described by King et al. (2010) along the south and south-east facing slopes of Green Lakes Valley, which is part of the Niwot Ridge Long Term Ecological Research (LTER) site in the Front Range of the Rocky Mountains, Colorado, USA. This area is typically covered with snow from October to June and the deepest snowfields do not melt fully until August. Precipitation averages 884 mm/yr with 94% falling as snow (Litaor et al. 2008). The study area extends over 2 km and is a distinct, well-defined landscape unit bound on the east by tundra, on the south by alpine lakes, glaciers and meadows, on the west by the continental divide and on the north by steep cliffs (King et al. 2010). The sampling area is primarily located within talus fields covered by granite blocks >1 m in diameter. Patches (up to 20 m in diameter) of soil with plant cover ranging from 0% to 100% are interspersed across this landscape.

Field sampling

In August and September 2015, we sampled a spatially-explicit grid of 98 1 m radius circular plots spaced at 50 m intervals with three clusters of plots spaced 5 m apart (King et al. 2010) (Appendix S1: Fig. S1). In each plot, we identified each species of vascular plants and recorded the presence of moss and lichen. Because plant species richness and plant density could co-occur, we recorded the number of stems of each plant species (clonal and cushion plants were counted as clumps rather than stems as representative of single genets) and the number of occurrences of mosses and lichens. To avoid disturbance to plants, we collected three soil cores per plot (3 cm in diameter and 4 cm in depth) away from plant stems and cushions, composited them into a plastic bag, gently homogenized them, and transported them on ice to the lab by the end of the day. Aliquots for DNA (0.3 g) and microbial enzyme assays (1 g) were subsampled immediately after returning from the field and subsequently frozen at – 20°C until further processing (Stenberg et al. 1998). The remaining soil was stored overnight at 4°C and aliquoted for an immediate extraction of microfauna (20 g), gravimetric soil moisture (5 g), extractions of C and N (5 g), and total C and N (%) (0.2 g). Aliquots for microbial C and N (5 g), water holding capacity (4 g), and pH (2 g) were stored frozen as above prior to analyses. Details of specific methods are below.

Belowground biota

Soil microbiota (bacteria, archaea, and eukaryotes) were determined using amplicon sequencing of 16S, 18S, and ITS gene markers. DNA was extracted from 0.3 g of soil using a PowerSoil DNA Isolation Kit according to the manufacture's protocol and amplified twice with the following primers: 515F/806R for Bacteria and Archaea (Fierer et al. 2012), ITS1-F/ITS2 for Fungi (McGuire et al. 2013), and 1391f/EukBr for Eukaryota (Amaral-Zettler et al. 2009). Multiplexing barcodes and PCR conditions were as described by the Earth Microbiome Project (http://www.earthmicrobiome.org/protocols-and-standards/) (Amaral-Zettler et al. 2009, Bellemain et al. 2010, Caporaso et al. 2012). Amplified samples were purified and normalized with SequalPrep Normalization Kit (Invitrogen Inc., Carlsbad, California, USA), combined into three single pools of either 16S, ITS, or 18S amplicon libraries, and sequenced on three lanes using identical Illumina technology (MiSeq2000, pair-end 2 × 300 bp) at the University of Colorado BioFrontiers sequencing facility. Because 0.3 g of soil is too small to accurately evaluate soil microfauna (e.g., nematodes), they were extracted from ~20 g soil subsamples using Whitehead trays for 24 h, captured on a 38 μm mesh sieve, transferred to PowerSoil DNA bead beating tubes (Porazinska et al. 2014) and processed for 18S sequencing as described above.

All raw amplicons (forward reads for 16S and reverse reads for 18S and ITS) were first evaluated for quality and trimmed when the mean quality score dropped below 25, generating reads of 230 bp for 16S, 160 bp for 18S, and 200 bp for ITS. All reads were subsequently demultiplexed and processed within the QIIME (ver.1.9.1) pipeline (Caporaso et al. 2010). All "soil-derived" reads were clustered "de novo" to OTUs at 97% similarity and "nematode-derived" reads at 99% (Porazinska et al. 2010) using UCLUST (Edgar 2010). Chimeras were removed using UCHIME (USEARCH7) (Edgar et al. 2011). To assign taxonomy, representative sequences of all 16S- and 18S-generated OTUs were BLAST-matched against the SILVA (ver.111) database (Pruesse et al. 2007) and ITS-generated OTUs against the UNITE database (Abarenkov et al. 2010). Singletons and doubletons as likely amplification/sequencing errors (Porazinska et al. 2012*a*) were removed prior to downstream analyses. As the ITS gene marker was used for fungal analyses, 18S fungal OTUs were removed from 18S datasets (non-fungal eukaryotes). Filtered 16S and 18S representative sequences were aligned using SINA (Pruesse et al. 2012) and phylogenetic trees built using FastTree (Price et al. 2009). Nematode OTUs clustered at 99% similarity were further reduced by using Head-Tail patterns (Porazinska et al. 2010) and only representative sequences of the "species-equivalent" OTUs were used for alignment and tree-building.

To ensure equal treatment of sequencing samples for statistical analyses, all samples were rarefied to an even depth: 16S to 6780 reads/sample for a total of 95 samples, 18S to 1,000 reads/sample for a total of 95 samples, and ITS to 5,000 reads/sample for a total of 94 samples. Instead of rarefying the nematode sequencing data, reads were converted to number of reads per standard unit of dry soil weight (20 g) for a total of 88 samples (Porazinska et al. 2010, 2012*b*).

Soil C and N pools and microbial activity

Soil C and N pools were measured as follows: dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and inorganic N (NH_4^+, NO_3^-) (μg/g dry soil) were extracted from 5 g of soil with 25 mL of 0.5 mol/L K_2SO_4 and shaken horizontally for 1 h at 250 rpm. Solutions were centrifuged for 3 min at 10,000 rpm (20124 G-force) and immediately filtered through 0.3 μm glass fiber filters (Advantec). Extracted DOC and TDN were analyzed using a Shimadzu total organic carbon analyzer equipped with a total dissolved nitrogen module (Shimadzu Scientific Instruments, Inc., Columbia, Maryland, USA), and inorganic N was assessed using Lachat QuickChem 8500 Flow Injection Analyzer (Lachat Instruments, Loveland, Colorado, USA) and Synergy 2 Multi-Detection Microplate Reader (BioTek Instruments, Inc., Vinooski, Vermont, USA). Microbial biomass C and N (μg/g dry soil) were estimated by placing 5 g of soil in 250 mL Erlenmeyer flasks to which 2 mL of liquid alcohol-free chloroform was added. The Erlenmeyer flasks were then sealed, fumigated for 24 h, vented for 1 h (until chloroform evaporated), and then followed with 0.5 mol/L K_2SO_4 extraction as described above. Microbial biomass N and C were calculated as the difference between TDN and DOC in fumigated and unfumigated samples. For total C and N (%), 4 g of soil were air-dried, ground manually in a pestle, ~50 mg packed into tin capsules, and combusted using a Thermo Finnigan Flash EA 1112 Series CHN analyzer (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA).

As an indicator of soil processes, we measured rates of seven microbial enzymes metabolizing organic C (α-glucosidase – AG, β-glucosidase – BG, β-xylase – BXYL, and cellobiosidase – CBH), N (N-acetylglucosamine – NAG and leucine aminopeptidase – LAP) and P (phosphatase – PHOS) macromolecules. Soil protocols that were optimized for these soils (Weintraub et al. 2007) involved processing soil slurries of 1 g of soil sample in 125 mL 0.5 mol/L sodium acetate buffer adjusted to pH 5.0 (approximating pH of soils) by adding 15–30 drops of glacial acetic acid, homogenized at 3000 rpm for 1 min using an Ultra-Turrax homogenizer (IKA Works Inc., Wilmington, North Carolina, USA), and incubated in the dark for 22 h at 13°C using controls and fluorescent substrates (based on 4-methylumbelliferone and 7-amino-4-methylcoumarin) (Weintraub et al. 2007, Sinsabaugh et al. 2008). Enzyme activity (nmol/h/g dry soil) (Bell et al. 2013) was evaluated by measuring fluorescence using a Synergy HT Multi-Detection Microplate Reader (BioTek, Vinooski, Vermont, USA).

Soil environmental measures

Soil moisture (%) was determined gravimetrically by determining weight loss of 5 g of soil after 48 h of drying at 60°C. To measure pH, 2 g of soil were suspended in 3 mL of ultrapure water (Honeywell) in 15 mL conical tubes and shaken horizontally for 1 h at 175 rpm. The slurry was transferred into a 10 mL glass beaker and pH was recorded when the reading stabilized (~15 s) using a calibrated Oakton benchtop pH meter (Oakton Instruments, Vernon Hills, Illinois, USA). Water holding capacity was determined gravimetrically by placing 4 g of soil in 15 mL bottom-meshed conical tubes, in which the soil was saturated with water and allowed to drain before being placed in an oven at 60°C for 15 h. Mean snow depth overlying each plot was calculated from snow depth surveys conducted in the Green Lakes Valley from 1997 to 2015. Annual snow surveys occur in May at peak snowpack along a grid of random points (mean $n = 483$) spaced ~50 m apart. We used kriging interpolation to create a continuous raster surface for each year, conferred the depths to all of our plot locations, and finally averaged the 22 yr to reflect long-term snow cover conditions. Elevation (m. a. s. l.) was recorded using a handheld Trimble GPS device with an error rate of 3 m.

Statistical analyses

To test for a positive relationship between diversity of plants (i.e., species richness) and soil groups (i.e., OTU richness and phylogenetic diversity), we constructed general linear models with richness (Chao 1) (Chao 1984) and phylogenetic distance (PDiv) (Faith 1992) (calculated in QIIME) as response variables of soil communities (Chao 1 richness was the only diversity metric calculated for fungi as ITS-generated sequences are difficult to align). Since plant density can co-occur with and affect soil biota irrespective of plant richness, it was also included in the models. Because

these relationships can be affected by environmental factors (i.e., pH, moisture, snow, and elevation), we extended the above models by a stepwise addition of these variables. They were added to the models in the order of their R^2 values with richness of soil groups (Appendix S1: Table S1A). The nature (positive or negative) of the association was deducted from the signs (+/−) of the coefficients in the models.

To test for positive relationships between plant/soil diversity and soil processes (e.g., different forms of C- and N-pools), we constructed general linear models with C and N (%C and %N, DON, DOC, TDN, inorganic N, microbial C and microbial N), CNP process rates (i.e., microbial enzyme activities) as response variables and plant richness/density, richness of soil groups, and environmental variables as predictive variables as described above. Variables were added to the models in the order of their R^2 values with C and N pools and processes (Appendix S1: Table S2).

All analyses were performed in R (R Core Team 2017) using the function 'lm' with forward selection in the 'Bestglm' package (McLeod and Xu 2011). Models were considered significant at $P < 0.05$ and the best models were selected by using the Akaike information criterion (AIC, Akaike 1974). To avoid potential collinearity among variables in any multiple regression model, we assessed variance information factors using the 'vif" function in the 'Car' package (Fox and Weisberg 2011). With no values >4, all selected models with their variables were retained. We confirmed the selected models using the 'dredge' function in the 'MuMIn' package (Bartoń 2018).

Results

Plant communities

Plant richness varied from 0 (no plants) to 27 species per plot (mean = 8, SD = 7). Plant species represented all major high-elevation plant groups, including forbs, grasses, sedges, rushes, and N_2-fixers (Appendix S1: Fig. S2A) and the number of these groups increased with an increase in total plant species richness (Appendix S1: Fig. S2B). Out of 75 species, seven (forbs: *Oxyria digina*, *Senecio fremontii and Castilleja occidnetalis*; grasses: *Festuca brachyphylla, Trisetum spicatum, and Poa albina*; sedges: *Carex pyrenaica*) were present across the entire plant richness spectrum and were consistently most abundant. *Deschampsia cespitosa* (grass) and *Geum rossi* (forb) were the next two most abundant species although they were absent in plots at the lowest plant richness (1–4 plant species) spectrum. As communities became more diverse and dense, the relative abundance of forb species increased while that of mosses declined. Nitrogen-fixing *Trifolium* species were present only in plots with high levels of plant species richness (>14). Although mycorrhizal, facultatively-mycorrhizal and non-mycorrhizal plants were present across the entire plant richness spectrum, relative abundance of mycorrhizal species increased and non-mycorrhizal decreased with higher plant richness. Plant richness was best explained by diminishing snow cover ($R^2 = 0.33$, $P = 6.78\text{E-}10$) and pH ($R^2 = 0.37$, $P = 3.93\text{E-}11$) and plant density by water holding capacity ($R^2 = 0.29$, $P = 9.15\text{E-}09$) and pH ($R^2 = 0.23$, $P = 7.56\text{E-}07$). In addition, plant density was significantly correlated with plant richness ($R^2 = 0.61$, $P = 2.20\text{E-}16$) (Appendix S1: Fig. S2C) and ranged from 0 to 762 stems per plot (mean = 96, SD = 119).

Relationship between richness of plants and soil biota

Both measures of diversity (richness and phylogenetic diversity) of all soil groups were positively associated with plant richness and density, but plant metrics consistently explained more variation in richness (Chao 1) of soil groups compared to phylogenetic diversity (PDiv) (Table 1, Appendix S1: Table S3). Although plant richness was generally a better predictor of diversity of soil groups than plant density, both variables were important predictors of belowground diversity (Table 1) and vice versa. The pattern of positive relationship with plant richness was observed for all soil groups: bacterial and non-fungal eukaryotic richness approximately doubled across the gradient (Fig. 1A,B), fungal richness showed the least change (Fig. 1C), and nematode richness was most pronounced (Fig. 1D) with an average of two nematode species in bare plots vs. 18 species at the opposite end of the plant richness gradient spectrum. The majority of environmental measures showed consistent significant positive (except for snow cover) relationships with richness of soil groups (Appendix S1: Table S1A); however, their predictive power was less than that of plant richness (Table 2, Appendix S1: Table S3). Overall, the best predictive models included a combination of both plant and environmental measures (Table 2, Appendix S1: Table S3). To ensure that plots free of vegetation did not disproportionately bias these results, all analyses were repeated without the unvegetated plots with unchanged patterns (Appendix S1: Tables S1B, S4).

A wide spectrum of soil taxa were identified in our plots (Appendix S1: Table S5). Among plant-dependent taxa, recognized bacterial mutualists/pathogens (Bull et al. 2010, Weir 2016) were represented by two orders: Rhizobiales (e.g., *Rhizobium*) ($R^2 = 0.40$, $P = 5.70\text{E-}12$, SI Fig. S3A) and Burkholderiales (e.g., *Burkholderia glumae*). Plant-dependent fungal taxa included dark septate endophytes (e.g., ascomycetous *Capronia* and *Phialocephala*) and glomeromycetous arbuscular mycorrhizae, which were represented by at least 16 genera that consistently increased in relative abundance with plant richness. With an exception of the fungus *Scutelospora*, all bacterial and fungal symbionts were typically absent in unvegetated plots and increased along the plant richness gradient. As expected, the abundance of pathogens, including obligate pathogens (e.g., *Fusarium oxysporium*) generally increased ($R^2 = 0.22$, $P = 2.02\text{E-}06$, Appendix S1: Fig. S3B) as plants became more species-rich. However, unlike mutualists, these pathogens were observed in soils where plants were completely absent. Free-living taxa varied across the landscape. Taxa recognized for their presence in more fertile soils (e.g., Spartobacteria, $R^2 = 0.43$, $P = 5.23\text{E-}13$, Appendix S1: Fig. S3C) or involvement in decomposition (e.g., Agaricomycetes, Appendix S1: Table S5) increased with plant richness. In contrast, photosynthetic microbes (e.g., Cyanobacteria and Chlorophyta, Appendix S1: Table S5) and cold- and snow-adapted (e.g., zygomycetous snow molds *Mortierella* spp., $R^2 = 0.20$, $P = 2.02\text{E-}06$, Appendix S1: Fig. S3D) decreased with plant richness.

TABLE 1. Parameters of the linear models predicting diversity (richness and phylogenetic diversity) of soil groups by plant variables (species richness and density).

	PR	PD	PR	PR + PD	PD
Bacteria					
Richness	**0.49**	0.29		0.50	
R^2					
Slope	110.65	4.80	114.55		−0.28
IC	87.60, 133.70	3.25, 6.34	77.25, 151.85		−2.39, 1.83
AIC	1533.13	1565.32		1535.06	
PDiv	**0.39**	0.19		0.40	
R^2					
Slope	2.53	0.10	3.00		−0.03
IC	1.88, 3.19	0.06, 0.14	1.93, 4.02		−0.09, 0.03
AIC	854.90	882.13		855.69	
Fungi					
Richness	**0.20**	0.08		0.21	
R^2					
Slope	10.45	0.38	13.51		−0.22
IC	6.12, 14.86	0.12, 0.64	6.48, 20.54		−0.61, 0.18
AIC	1200.72	1213.46		1201.50	
Non-fungal Eukaryotes					
Richness	**0.49**	0.24		0.50	
R^2					
Slope	15.43	0.60	18.05		−0.19
IC	12.13, 18.73	0.36, 0.82	12.78, 23.31		−0.48, 0.11
AIC	1137.70	1174.74		1138.07	
PDiv	0.31	0.14		0.31	
R^2					
Slope	0.67	0.03	0.80		−0.01
IC	0.46, 0.88	0.01, 0.04	0.47, 1.14		−0.03, 0.01
AIC	625.54	645.29		626.49	
Nematodes					
Richness	0.45	0.42		**0.49**	
R^2					
Slope	0.77	0.04	0.48		0.02
IC	0.58, 0.96	0.03, 0.05	0.20, 0.76		0.01, 0.04
AIC	541.18	545.17		535.88	
PDiv	**0.42**	0.28		0.42	
R^2					
Slope	0.09	0.003	0.08		0.001
IC	0.65, 0.11	0.003, 0.005	0.04, 0.11		−0.001, 0.003
AIC	180.83	199.18		182.39	

Notes: PR = plant richness (number of plant species), PD = plant density (number of stems). Diversity of soil groups included: richness measured by Chao 1 and PDiv by Faith's phylogenetic diversity. All R^2 values indicative of the predictive power of the models were significant at $P < 0.05$ (but see Table 2 for details). IC = slope's confidence intervals at 2.5% and 97.5% (calculated using the 'confin' function). The best models were selected using AIC criterion and are indicated in bold.

Relationships between plant and microbe richness and soil C and N

Soil C and N pools (e.g., % total C and N, microbial C and N, Fig. 2) were generally very low. The size of C and N pools (except for NO_2^-/NO_3^- and NH_4^+) significantly increased with increasing plant richness and density, paralleling the patterns of the belowground community (Appendix S1: Table S2). Water holding capacity and moisture were the most significant and the most predictive environmental factors of C and N soil pools. However, the most predictive power came from models that incorporated plant (both richness and density), soil biotic, and environmental factors (Table 3). Microbial enzyme activities associated with processing of C-, N-, and P-compounds corroborated these results. Enzymatic activities were low (or barely detectable) where plants were absent or barely present, and generally highest where plant communities were most complex. Although plant richness and density were significant predictors of nearly all enzyme activities (Appendix S1: Table S2), microbial C and/or water holding capacity provided the best explanatory power (Table 3). From all tested microbial enzymes, phosphatase (mean = 156, SD = 219 $nmol \cdot h^{-1} \cdot g^{-1}$ dry soil) and β-glucosidase (mean = 112, SD = 164 $nmol \cdot h^{-1} \cdot g^{-1}$ dry soil) showed the highest activities and leucine aminopeptidase (mean = 1, SD = 1 $nmol \cdot h \cdot g^{-1}$ dry soil) the lowest.

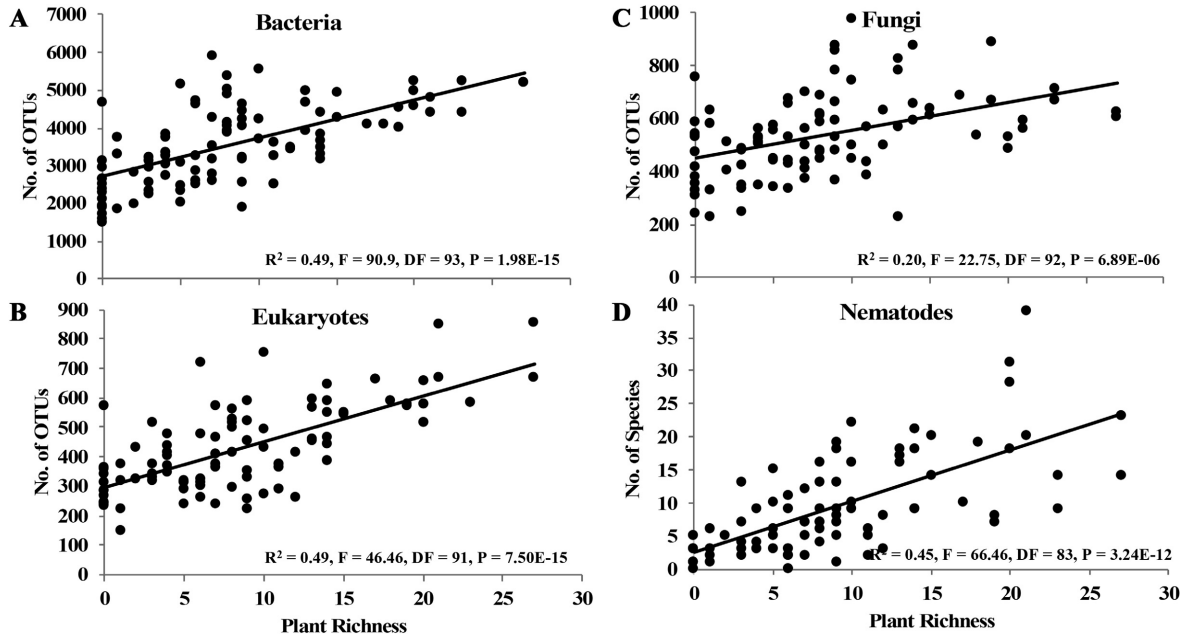

FIG. 1. Relationship between plant richness and richness (Chao 1) of soil groups as predicted by linear regression models. (A) Bacteria (16S), (B) Non-fungal eukaryotes (18S), (C) Fungi (ITS), and (D) Nematodes (species based on 18S Chao 1).

TABLE 2. Forward-selection linear models predicting soil richness (Chao 1) with the best plant variable only, with the best biogeochemical variable only, and a combination of both. For relationships with phylogenetic diversity see Appendix S1: Table S3.

	R^2	F	df	P
Bacteria				
+PR	0.49	90.90	1, 93	1.98E-15
+pH	0.51	92.2	1, 93	6.68E-16
+pH, +PR, -snow	0.70	71.58	3, 91	<2.20E-16
Fungi				
+PR	0.20	22.75	1, 92	6.88E-06
-snow	0.14	15.44	1, 92	1.65E-04
+PR, -snow	0.22	13.04	2, 91	1.05E-05
Non-fungal eukaryotes				
+PR	0.49	86.66	1, 91	7.50E-15
+pH	0.34	46.15	1, 91	1.10E-09
+WHC, +pH, +PR	0.55	35.49	3, 89	3.52E-15
Nematodes				
+PR	0.45	66.46	1, 83	3.24E-12
+WHC	0.44	65.23	1, 83	4.58E-12
+WHC, +pH, -snow, +PR	0.64	35.13	4, 80	<2.20E-16

Notes: Variables were added to models based on best pairwise R^2 values in Table 1 and SI Table 1A. PR = plant richness, pH = soil pH, WHC = water holding capacity, Snow = 1995–2017 average snow depth cover, +/– = positive/negative relationship inferred from the signs of coefficients.

DISCUSSION

Few studies have provided empirical evidence supporting theoretical expectations for correlations between richness of plants and soil biota and ecosystem functioning in non-manipulated natural communities (Wardle 2006, 2016). With the majority of studies (e.g., Porazinska et al. 2003, Wardle 2006, Wardle et al. 2012, Lekberg et al. 2013, Tedersoo et al. 2014, Prober et al. 2015) finding little relationship between richness of plants and soil groups, the leading conclusion (counter to theoretical expectations) is that the link between plant communities and soil communities in terms of richness is, at best, weak. In contrast, we observed consistently strong positive relationships between the richness of plants and all investigated soil groups including bacteria and fungi, as well as organisms typical of higher trophic levels of soil foodwebs (e.g., nematodes). Because of these strong, consistent positive relationships, we suggest that an appropriate question is not whether these relationships are present across different ecosystems, but whether they can be detected. It is possible that the ability to detect plant-soil diversity/function relationships depends largely on the properties of the soil environment, and specifically the amount of organic carbon reflective of the presence/absence of the legacy of plant-soil inputs, but admittedly this needs to be explicitly tested in experimental studies.

Soil communities exist in a matrix of complex biotic and abiotic factors continuously interacting with each other (Hunt et al. 1987). How these interacting factors influence soil communities and to what extent they affect them has remained elusive, partially because studies have been generally conducted in relatively complex environments with not only contemporary but also past carbon inputs. These legacy effects are evidenced by the presence of substantial pools of carbon in the form of detritus (Moore et al. 2004). In successional systems where these legacy soil C pools are well-established (e.g., in grasslands), plants and soil biota can interact indirectly by acquiring needed resources from these legacy pools. In contrast, in early successional soil where legacy C is largely absent, plants and soil biota may have to interact more directly. Because legacy C pools in the high-alpine soils are minimal, plants and soil biota may depend on each other by immediately utilizing the resources

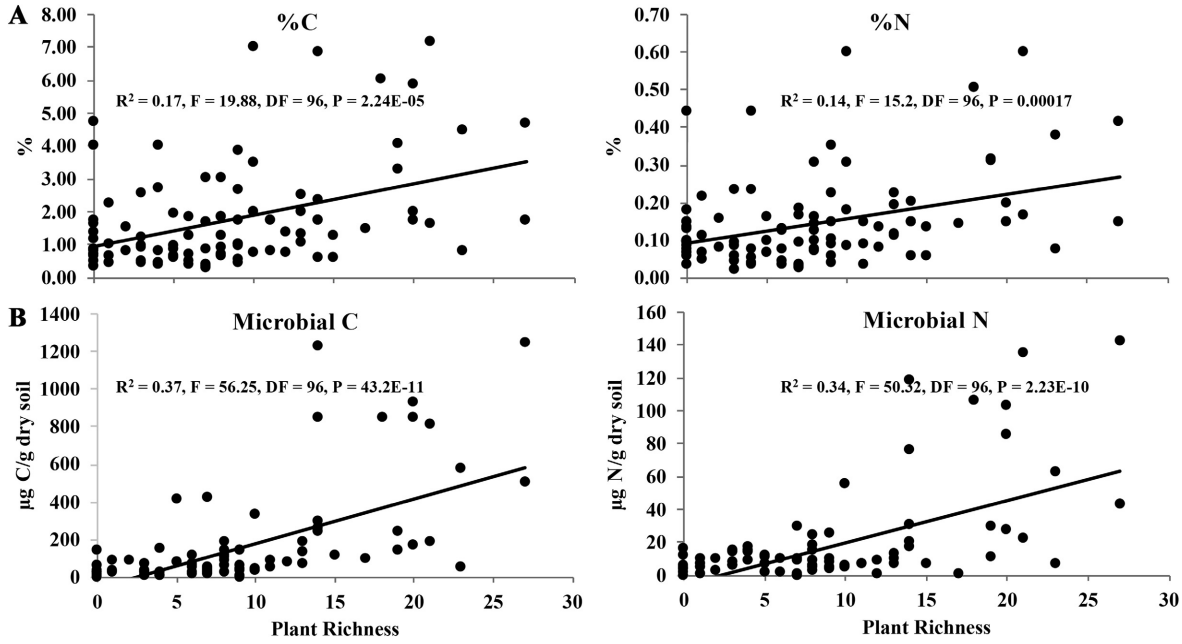

FIG. 2. Examples of carbon and nitrogen pools predicted by linear regression models along plant richness gradient. (A) % C and N; (B) Microbial C and N.

they produce. In addition, the constrained growing season length (1–3 months) at this site perhaps reinforces this coupling. But as legacy C builds up, plants and soil biota should gradually transition from direct (immediate resource) to more indirect (through legacy resources) interactions. In other words, in soils where legacy C is still minimal (as in our system), taxonomic richness reflects this direct coupling between plants, soil biota, and processes. But in soils where legacy C is more abundant (e.g., in grassland and forests), such coupling may be obscured. While here we have focused predominatly on plant richness (as the most consistent predictive variable), we recognize that in this early successional systems richness cannot be completely teased apart from density. Since density strongly correlated with richness, both variables likely play a role in the effects on soil biota. How different plant community properties (including composition) are drivers of specific soil groups at this site are discussed elsewhere (e.g., Bueno de Mesquita et al. 2018*b*).

It is important to note that, although generally consistent in direction, the strength of the relationships between plant richness and soil groups varied (e.g., fungal richness was the least correlated with plant richness). Within individual soil groups, positive shifts were driven by specific relevant taxa rather than all taxa responding in a concerted fashion. For instance, within bacteria, Spartobacteria (recognized to prefer fertile soils, Bergmann et al. 2011) and Rhizobiales (considered plant dependent N-fixers and pathogens, Kersters et al. 2006) were among key taxa contributing to the observed diversity patterns along the plant richness gradient. Within fungi, Glomeromycota previously shown to increase P uptake by alpine plants (Mullen and Schmidt 1993) and versitile heterotrophs in the Agaricales (Hibbett et al. 2014) were most significant. All of these taxa that were positively associated with plant diversity were likely indicative of increasing availability of hosts, food resources, and niches that together reflected slowly improving soil organic carbon.

Because soil organisms are functionally diverse, including taxa that don't rely on plants, their more neutral (or even negative) relationships with plants would diminish the overall strength of the positive coupling observed in the present study. Indeed, photosynthesizing taxa, including cyanobacteria and Chlorophyta (green algae), collectively showed patterns opposite to those of plant communities, likely driven by competition with plants for light and soil nutrients. In addition, fungi specifically adapted to within- and under-snow niches, such as *Rhodotorula* (Connell et al. 2014, de Garcia et al. 2014) and *Mortierella* spp., a zygomycetous "snow mold" (Schmidt et al. 2008*b*), both decreased in abundance along the plant richness gradient. Given this wide spectrum of soil biota life-history strategies, the strength of positive above-belowground relationships will vary and the key to understanding these relationships is a detailed knowledge of specific taxa.

Unvegetated soils at our site are mostly made of parent material with organic carbon levels that are very low (~1%, King et al. 2008) compared to vegetated soils on Niwot Ridge (>20%, Fisk 1995), but higher than levels in the most extreme early successional soils in alpine environments (~0.1%, Nemergut et al. 2007). However, with increasing presence of plants and their soil biota along the plant richness gradient, we observed a buildup of C and N pools (both microbial and total) and improved water holding capacity under the most diverse plant communities. Also, the accumulation of organic matter and microbial biomass likely improved the ability of soil to retain nutrients and thus make soil pH more basic. Since plants and soil microbes are the predominant sources of organic matter, all biotic and abiotic components were correlated along the plant richness gradient. In fact, unlike in other studies (e.g., Prober et al. 2015,

TABLE 3. Forward-selection linear models predicting C and N pools and processes with best plant variable only, best soil diversity only, best biogeochemical only, and a combination of all (if the model was better).

	R^2	F	df	P
Soil pools				
Carbon				
%C				
+PD	0.19	19.88	1, 96	2.24E-05
+Euk	0.14	14.92	1, 91	0.0002
+WHC	0.47	86.53	1, 96	4.70E-15
+WHC, +PR, +PD, +Bac	0.49	21.57	4, 90	8.33E-06
MicC				
+PD	0.42	70.42	1, 96	4.18E-13
+Euk	0.21	24.81	1, 91	2.94E-06
+WHC	0.67	192.4	1, 96	<2.20E-16
+WHC, +PR, +PD, +Bac, +Euk	0.87	116.60	5, 89	<2.20E-16
DOC				
+PD	0.17	19.10	1, 96	3.14E-05
+Euk	0.19	19.88	1, 91	2.35E-05
+WHC	0.35	51.07	1, 96	9.83E-08
+WHC, +Euk	0.39	28.55	2, 90	2.20E-10
Nitrogen				
%N				
+PD	0.16	18.29	1, 96	4.47E-05
+Euk	0.19	21.70	1, 91	1.07E-05
+WHC	0.39	60.17	1, 96	9.25E-12
MicN				
+PD	0.41	65.52	1, 96	1.79E-12
+Euk	0.25	29.72	1, 91	4.25E-07
+WHC	0.58	134.00	1, 96	<2.2E-16
+WHC, +PD, +Euk	0.73	59.16	4, 88	<2.2E-16
TDN				
+PD	0.09	9.81	1, 96	0.002
+Euk	0.15	15.56	1, 91	0.0002
+WHC	0.35	50.97	1, 96	1.79E-10
+WHC, +Euk	0.34	23.59	2, 90	5.80E-09
NO_2^-/NO_3^-				
PR, PD	ns	–	–	–
Bac, Fung, Euk	ns	–	–	–
+WHC	0.23	29.22	1, 96	4.70E-07
+WHC, -pH	0.40	31.39	2, 95	3.22E-11
NH_4^+				
+PD	0.05	5.15	1, 96	0.03
+Euk	0.07	6.88	1, 91	0.01
+WHC	0.50	96.99	1, 96	3.15E-16
IN				
PR, PD	ns	–	–	–
Bac, Fung, Euk	ns	–	–	–
+WHC	0.46	79.98	1. 96	2.77E-14
Enzyme activities				
Carbon-associated				
AG				
+PD	0.06	5.94	1, 96	0.02
+Euk	0.05	6.41	1, 96	0.03
+MicC	0.35	51.60	1, 96	1.46E-10
+WHC	0.28	37.31	1, 96	1.76E-08
+MicC, +WHC, +PD	0.39	19.64	3, 93	6.03E-10
BG				
+PD	0.11	11. 91	1, 96	0.001
+Euk	0.10	9.76	1, 91	0.002
+MicC	0.42	68.30	1, 96	7.81E-13
+WHC	0.37	60.63	1, 96	8.03E-12
+MicC, +WHC	0.44	37.25	2, 94	1.21E-12
BXYL				
+PD	0.09	9.03	1, 96	0.003
+Euk	0.05	4.67	1, 91	0.03
+MicC	0.32	45.81	1, 96	1.03E-09
+WHC	0.23	28.10	1, 96	7.34E-07
+MicC, +WHC, +PD	0.43	17.37	4, 91	1.28E-10
CBH				
+PD	0.06	6.57	1, 96	0.01
Bac, Fung, Euk	ns	–	–	–
+MicC	0.32	43.83	1, 96	2.05E-09
+WHC	0.25	32.52	1, 96	1.30E-07
Nitrogen-associated				
LAP				
PR, PD	ns	–	–	–
Bac, Fung, Euk	ns	–	–	–
+MicC	0.09	9.33	1, 96	0.002
+WHC	0.13	14.69	1, 96	2.26E-04
NAG				
+PD	0.12	13.26	1, 96	0.0004
Bac, Fung, Euk	ns	–	–	–
+%C	0.26	30.01	1, 96	1.08E-07
+moisture	0.16	17.81	1, 96	5.53E-05
Phosphorus-associated				
PHOS				
+PD	0.11	12.19	1, 96	0.0007
Bac, Fung, Euk	ns	–	–	–
+MicC	0.37	55.87	1, 96	3.61E-11
+WHC	0.31	44.05	1, 96	1.90E-09
+MicC, +WHC	0.38	21.29	2, 95	1.03E-10

Notes: Variables were added to models based on pairwise R^2 values in SI Table 2. Carbon pools: %C = percent of total carbon, MicC = microbial carbon, DOC = dissolved organic carbon. Nitrogen pools: %N = percent of total nitrogen, MicN = microbial nitrogen,, TDN = total dissolved nitrogen, IN = inorganic nitrogen ($NO_2^-/NO_3^-/NH_4^+$). Enzyme activities associated with carbon: AG = α-glucosidase, BG = β-glucosidase, BXYL = β-xylase, CBH = cellobiosidase; with nitrogen: LAP = leucine aminopeptidase, NAG = N-acetylglucosamine; and with phosphorus: PHOS = phosphatase. WHC = water holding capacity, PR = plant richness, PD = plant density, Bac = 16S richness (Chao 1), Euk = 18S richness (Chao 1) of non-fungal eukaryotes, Fung = ITS richness (Chao 1), pH = soil pH. +/– = positive/negative relationship inferred from the signs of coefficients.

Yuan et al. 2017), most plant, soil taxa, and environmental parameters were positively correlated, and the best models for predicting C and N pools included variables from all three categories. Given that the buildup of C and N pools along the plant richness gradient remained generally marginal (in comparison to other ecosystems, e.g., grasslands), the absence of legacy pools in these soils in their early stages of development provided a suitable background for this subtle accumulation of C and N pools to be detected. In contrast, in well-developed soils with long histories of plant/soil biota inputs (legacy C), this change would likely be unnoticed. In addition, although the release of nutrients through decomposition may be partially taken back up by plants and soil communities, a significant fraction may also be lost

in runoff due to the lack of legacy C (Schmidt et al. 2015), likely feeding back to strengthening of the plant-soil biota coupling in this system.

We found further support for positively coupled above-belowground linkages from our measurements of extracellular enzymes. Most enzymes, as expected for soils with a marginal quantity of polymeric compounds, showed comparatively very low activities, particularly in bare and barely vegetated soils, but increased significantly in more vegetated soils. This pattern was consistent for all the enzymes. The relatively higher activities of phosphatase and β-glucosidase indicate that microbes in these high alpine soils are mainly limited by phosphorus and carbon (King et al. 2008) and that inorganic nitrogen coming from atmospheric N-deposition and free-living N-fixers (e.g., the dominant cyanobacterium, *Microcoleus* sp.) might be less limiting. However, as vascular plant richness and density increase over time, nitrogen may become more limiting relative to phosphorus and carbon (Bueno de Mesquita et al. 2017).

Although the most predictive factors for enzyme activities were microbial C and water-holding capacity, these factors are directly linked back to plant communities as the main sources of organic matter. These results emphasize the critical role of plants and plant inputs in initial structuring of soil communities, either as hosts for plant-dependent taxa or through exudates and litter inputs for free-living taxa. When legacy carbon is absent, mineralized nutrients, unless immediately re-assimilated by plants or microbes, can be lost through leaching, suggesting that microbial biomass may provide a critical initial mechanism for temporary nutrient storage. In contrast, in soil with well-established legacy C, nutrients can be retained within accumulated soil organic matter and accessed by plants and soil biota independently of each other (e.g., asynchronized in space and time) appearing as largely uncoupled, but in reality they are coupled indirectly via legacy C pools and consequently difficult to observe.

Conclusion

We found consistent evidence of positive relationships between plant richness (and density), soil biodiversity, and function across a high-elevation landscape. We attribute our ability to detect this coupling predominantly to the unique gradient of plant richness and density across this alpine landscape. We suggest that the absence of legacy C facilitates more direct interactions between contemporary resources and biota and consequently a stronger and more apparent coupling between plant and soil biodiversity and ecosystem functioning. As plants keep moving up and becoming a more consistent component of high-alpine landscapes, we expect the coupling to become eventually less apparent and thus consistent with previous studies where legacy pools of resources are more concentrated. In the meantime, carbon deficient oligotrophic ecosystems, especially high-alpine ecosystems, should provide excellent testing grounds for answering critical questions about the dynamics of relationships between plants, soil groups, and soil processes.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2420/suppinfo

Data Availability

All data, including raw sequences and mapping files, processed OTU tables and OTU's representative sequences, and all associated metadata, are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.tn39dc6