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Environmental filtering controls soil biodiversity in wet tropical ecosystems

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ABSTRACT

The environmental factors controlling soil biodiversity along resource gradients remain poorly understood in wet tropical ecosystems. Aboveground biodiversity is expected to be driven by changes in nutrient availability in these ecosystems, however, much less is known about the importance of nutrient availability in driving soil biodiversity. Here, we combined a cross-continental soil survey across tropical regions with a three decades' field experiment adding nitrogen (N) and phosphorus (P) (100 kg N $ha^{-1}y^{-1}$ and 100 kg P $ha^{-1}y^{-1}$) to Hawai'ian tropical forests with contrasting substrate ages (300 and 4,100,000 years) to investigate the influence of nutrient availability to explain the biodiversity of soil bacteria, fungi, protists, invertebrates and key functional genes. We found that soil biodiversity was driven by soil acidification during long-term pedogenesis and across environmental gradients, rather than by nutrient limitations. In fact, our results showed that experimental N additions caused substantial acidification in soils from Hawai'i. These declines in pH were related to large decreases in soil biodiversity from tropical ecosystems in four continents. Moreover, the microbial activity did not change in response to long-term N and P additions. We concluded that environmental filtering drives the biodiversity of multiple soil organisms, and that the acidification effects associated with N additions can further create substantial undesired net negative effects on overall soil biodiversity in naturally tropical acid soils. This knowledge is integral for the understanding and management of soil biodiversity in tropical ecosystems globally.

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1. Introduction

Tropical forests play important roles in sustaining human wellbeing and maintaining biodiversity and ecosystem functioning worldwide (Achard et al., 2002; Edwards et al., 2014; Souza et al., 2021). Tropical forests are often described as the lungs of the Earth, because of the amount of CO_2 they exchange with the atmosphere. These forests are also a refuge for biodiversity, and are home to millions of people. However, tropical forests are also known to contain very old and weathered soils limited by nutrient availability, especially phosphorus (P) (Vitousek and Farrington, 1997; Vitousek et al., 2010). Although P is often regarded as one of the major limiting factors to tropical forest productivity (Chadwick et al., 1999; Turner et al., 2018; Johnston et al., 2019), much less is known about the importance of other environmental factors (i.e., availability of other nutrients, soil acidification) at explaining soil biodiversity in tropical forests.

Belowground organisms (i.e., bacteria, fungi, protists and invertebrates) constitute a large fraction of biodiversity in terrestrial ecosystems worldwide (Bardgett and van der Putten, 2014; Wagg et al., 2014; Zhu et al., 2021). These organisms play critical roles in regulating foundational ecosystem processes, such as plant productivity and competition, nutrient cycling, carbon (C) sequestration, soil stoichiometry, and organic matter decomposition (Soliveres et al., 2016; Delgado-Baquerizo et al., 2017a, 2020a; Guerra et al., 2020). Nitrogen (N) and P availability influence soil biodiversity of many ecosystems (Delgado-Baquerizo et al., 2017b; Ding et al., 2019; Clausing et al., 2020), however, we still know little of the belowground biodiversity of tropical forests, which are often underrepresented in global surveys (Fierer, 2017; Bakker et al., 2019; Cameron et al., 2019; Delgado-Baquerizo et al., 2019). We know belowground organisms can be highly responsive to N and P additions in terrestrial ecosystems (Reed et al., 2011; Leff et al., 2015; Zhou et al., 2020), yet critical knowledge gaps exist in the main environmental factors controlling the changes in soil biodiversity along resource gradients from tropical forests.

Here, we used a field survey in thirteen tropical ecosystems from four continents, and a three decades N and P fertilization experiment being conducted in two contrasting stages of a long-term chronosequence from Hawai'i (300 vs. 4,100,000 years) to investigate the role of nutrient limitations in driving soil biodiversity, including bacteria, fungi, protists, invertebrates and key functional genes (antibiotic resistance genes and fungal groups). Soil chronosequences are considered to be model systems for investigating the effects of nutrient depletion on biodiversity and ecosystem function (Wardle et al., 2004, 2008; Tarlera et al., 2008). Plant productivity is known to be limited by N at the youngest site in this chronosequence, while both plant productivity and litter decomposition are enhanced by P additions on the oldest site in the chronosequence (Vitousek and Farrington, 1997; Hobbie and Vitousek, 2000). Further, short-term additions of N and P (added with C) did stimulate microbial growth, with N having larger effects at the youngest site and P larger effects at the oldest site (Reed et al., 2011). Thus, sustained additions of limiting nutrients (N and P) could reduce the diversity but increase abundance of belowground organisms in these sites by stimulating the growth of a few opportunistic species that suppress the growth of sensitive species (Evans and Wallenstein, 2014; Cui et al., 2020). This phenomenon is widely observed for plants in N-limited ecosystems, where N additions can enhance productivity but reduce diversity (Clark and Tilman, 2008; Bai et al., 2010; Liu et al., 2019) – a specific example of the well-known "paradox of enrichment" (Rosenzweig, 1971). However, the influence of nutrient limitations on soil biodiversity is far less understood. Belowground diversity and function are known to change as soils develop from centuries to millennia (Wardle et al., 2009; Walker et al., 2010; Delgado-Baquerizo et al., 2019). We aim to address a fundamental question in tropical forests: what is more important, acidification or nutrient availability, when it comes to driving soil biodiversity in tropical forest ecosystems? Unlike young soils, well-developed soils from tropical and temperate forests are expected to be acid, show higher soil N to P ratios (Laliberté et al., 2013, 2014; Delgado-Baquerizo et al., 2020b), and support a lower soil biodiversity (Delgado-Baquerizo et al., 2019). Improving our understanding of the main environmental factors controlling soil biodiversity in tropical forests is crucial for making better predictions of the role of nutrient availability and acidification in driving soil biodiversity.

2. Materials and methods

2.1. Study sites

2.1.1. Hawai'i chronosequence experiment

The experiment was conducted at the Hawai'ian Islands (19°53′-22°05′N, 154°81′-160°22′W), which represent a soil chronosequence (the Long Substrate Age Gradient; LSAG) with soils that differ markedly in the age of their underlying substrate, from 300 to 4,100,000 years old. The forest study sites have a similar climate, with a mean annual temperature of 16 °C, and mean annual precipitation of approximately 2500 mm (Chadwick et al., 1999). The soil substrate is basaltic rock admixed with tephra and pumice, and the initial chemistry is oceanic basalt and thus relatively consistent across sites (Wright and Helz, 1987). The native tree *Metrosideros polymorpha*, is the dominant species, making up 80–88% of each forest's basal area (Kitayama and Mueller-Dombois, 1995). Several other species, including *Morella faya, Vaccinium calycinum*, *Hedychium gardnerianum,* and *Dicranopteris linearis* are also present in most sites along the chronosequence (Kitayama and Mueller-Dombois, 1995).

The fertilization experiments of the 300 and 4,100,000 year sites were established in 1985 and 1991, respectively. Three replicated plots measuring from 50 × 50 m were created, with each plot containing several trees. The treatments include Control (CK, no fertilizer application), N addition (100 kg N $ha^{-1}y^{-1}$, initially half as urea and half as ammonium nitrate; since 2002, all urea), P addition (100 kg P $ha^{-1}y^{-1}$ as triple superphosphate), and combination of N and P additions. Fertilizers were applied once in July 1993, and twice every year (every 6 months) thereafter (Vitousek and Farrington, 1997) until 2006; since then applications have occurred every other year. The present study was conducted after 26 and 32 years (2017) of the long-term N and P additions.

In 2017, soil samples were collected according to a standardized sampling protocol as described by Delgado-Baquerizo et al., 2020a. We collected three composite topsoil samples from five 0–10 cm deep soil cores under the dominant tree, which meant that 15 cores were collected in each treatment plot. After soil sampling, composite samples were sieved over a 2 mm mesh. The soils were separated into two parts. One part was air-dried and used for soil physicochemical analysis. The second part was immediately stored at −20 °C for amplicon sequencing analysis. To homogenize soils and obtain a representative sample for sequencing analysis, 10 g soil from the composite samples were freeze-dried using a mortar and liquid N.

2.1.2. Environmental gradient soil survey

Topsoils were collected from thirteen wet tropical ecosystems in four countries (USA, India, Nigeria and Brazil, Fig. S1) between 2016 and 2018 according to a standardized sampling protocol as described by Delgado-Baquerizo et al., 2020a. These ecosystems include natural and urban greenspaces, and different vegetation types, from grasslands to forests. The wet tropical ecosystems were defined as Köppen-Geiger climate classification (Beck et al., 2018). -Three composite soil samples (from five soil cores each) were collected under the most common environments (vegetation and open areas between plant canopies) found at each plot. A total of 39 composite soil samples were included in this study. After field collection, each composite soil sample was divided into two sub-samples - one sub-sample was immediately frozen at −20 °C for molecular analyses while the other sub-sample was air-dried for chemical analyses.

2.2. Soil properties analyses

Soil pH was determined in a suspension of soil and deionized water (1:2.5 mass:volume), using a pH electrode. Soil available N (ammonium and nitrate) and available P concentrations were determined colourimetrically using K_2SO_4 and bicarbonate extracts, respectively, as described by Delgado-Baquerizo et al., 2013. Soil total N and P concentrations were obtained after digestion with sulfuric acid for 3 h at 415 °C, using a SKALAR San++ Analyzer (Skalar, Breda, The Netherlands) (Maestre et al., 2012). Total soil organic C was determined colourimetrically using a mixture of potassium dichromate and sulfuric acid (Anderson and Ingram, 1993).

2.3. Microbial biomass and activity measures

Soil microbial biomass was estimated using phospholipid fatty acids (PLFAs) extracted from freeze-dried soils (Buyer and Sasser, 2012). The extracted fatty acid methylesters were analyzed using a DB-5 column in an Agilent Technologies 7890B gas chromatography–mass spectrometry (GC–MS) system (Agilent Technologies, CA, USA). The biomarkers used to indicate total bacterial biomass include i15:0, a15:0, 15:0, i16:0, 16:1ω7, 17:0, i17:0, a17:0, cy17:0, 18:1ω7 and cy19:0, and the biomarker 18:2ω6 was selected to indicate total fungal biomass (Frostegård and Bååth, 1996). Total microbial biomass is the sum of all bacterial and fungal biomarkers, and the eukaryotic biomarker 18:1ω9. Soil microbial activity (i.e., soil basal, glucose-, and lignin-induced respiration) was assessed by the Microresp® approach according to Campbell et al. (2003). Further details regarding the determination of soil basal, glucose-, and lignin-induced respiration can be found in Delgado-Baquerizo et al., 2017c.

2.4. Soil molecular analyses

The richness and community composition of bacteria, fungi, protists, and invertebrates were determined via amplicon sequencing using the Illumina MiSeq platform. Soil DNA was extracted using the Powersoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. The bacterial 16S genes were sequenced using the primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2012). The eukaryotic 18S rRNA genes were sequenced using the primer pair Euk1391f (5′-GTACACACCGC-CCGTC-3′) and EukBr (5′-TGATCCTTCTGC-AGGTTCACCTAC-3′) (Ramirez et al., 2014). Bioinformatics processing was performed using a combination of QIIME (Caporaso et al., 2010), USEARCH (Edgar, 2013) and UNOISE3 (Edgar, 2016). Sequences were clustered into soil phylotypes (i.e., Operational Taxonomic Units; OTUs) using a 100% identity level. Before calculating the richness of soil organisms, the OTU abundance tables were rarefied at 12,545 (bacteria via 16S rRNA gene), 6472 (fungi via 18S rRNA gene), 1299 (protists via 18S rRNA gene) and 348 (invertebrates via 18S rRNA gene) sequences per sample, respectively, to ensure even sampling depth within each belowground group of organisms. Note that protists are defined as all eukaryotic taxa, except fungi, invertebrates (Metazoa), and vascular plants (Streptophyta). We used the online FUNguild to determine the functional groups of fungi (i.e., saprotroph, plant pathogen, and arbuscular mycorrhizal fungi (AMF)) (Nguyen et al., 2016). We used only highly probable and probable guilds for these analyses. The abundance of 285 unique antibiotic resistance genes (ARGs) encoding resistance to all of the major categories of antibiotics was determined from soil samples using the Wafergen Smartchip high throughput qPCR (Hu et al., 2018).

2.5. Statistical analyses

Three-way ANOVA was used to test the individual and interactive effects of N and P addition and soil age on the richness of soil organisms (i.e., bacteria, fungi, protists, invertebrates, ARGs, saprotroph, and AMF), soil properties (i.e., pH, available N and P, total soil N and P, and total soil organic C), microbial activity (i.e., soil basal, glucose-, and lignin-induced respiration), and bacterial and fungal biomass. Before conducting each ANOVA analyses, the normal distribution was tested using the Shapiro-Wilk test. The data were square-root or log-transformed when necessary. Generalized linear mixed models (GLMMs) were employed to analyse the relationships between belowground biodiversity (i.e., bacteria, fungi, protists richness) and soil pH by accounting for random effects of country/site from four countries. Variation partitioning analysis and Monte Carlo permutation tests (999 permutations) were used to quantify the relative importance and significance of N and P addition, soil age, soil pH, available N, and soil total N:P ratio on the community composition of bacteria, fungi, protists, invertebrates and ARGs. The effects of N and P addition and soil age on the community composition of soil organisms were determined using PERMANOVA (999 permutations, Adonis function) and visualized by non-metric multidimensional scaling (NMDS, based on the Bray-Curtis distance matrices). Structural equation modelling (SEM, Grace, 2006) was used to quantify the direct and indirect effects of N and P addition and soil age on the richness of bacteria, fungi and protists of the Hawai'i experiment. We used the Chi-square test (χ^2; $0 \leq \chi^2/df \leq 2$ and $0.05 < P \leq 1.00$), and the root mean square error of approximation (RMSEA; $0 \leq$ RMSEA ≤ 0.05 and $0.10 < P \leq 1.00$) to confirm the goodness of fit for the models (Delgado-Baquerizo et al., 2020c). These SEM analyses were conducted using the software IBM SPSS AMOS 20 (Chicago, IL: Amos Development Corporation). In addition, we used "piecewiseSEM" (Lefcheck, 2016), "nlme" and "lme4" packages (Bates et al., 2017) to quantify the direct and indirect effects of mean annual precipitation (MAP), mean annual temperature (MAT), and soil properties (soil pH, available N and P, and total soil organic C) on the richness of bacteria, fungi, and protists by accounting for random effects of country/site from four countries. The Fisher's C test (when $0 \leq$ Fisher's C/df ≤ 2 and $0.05 < P \leq 1.00$) was used to confirm the goodness of the modelling results (Liu et al., 2020). The prior model was constructed as used in Maestre et al. (2015). None of the SEM analyses included plant composition data. The responses of the common taxa (top 10% relative abundance of bacterial, fungal and protists taxa, OTU number >1000) and all taxa of invertebrates, and ARGs, (OTU number <1000) to N and P additions were determined using Spearman correlation (Delgado-Baquerizo et al., 2018). The responsive (i.e., opportunistic and sensitive) and non-responsive taxa were further defined by the positively, negatively and not responding taxa to N and P additions using Spearman correlation, respectively. The taxa increasing in relative abundance to N or P addition compared to the no fertilizer application plots were called opportunistic taxa to N or P addition, and the taxa decreasing and not changing in relative abundance were called sensitive and non-responsive taxa, respectively. The relationships between bacteria, fungi and protists, and soil pH were also tested using Spearman correlation. All of these statistical analyses and visualisations were performed using the *datasets*, *RColorBrewer*, *lavaan*, *vegan* and *ggplot2* packages in the R v.3.5.3 software (https://www.r-project.org).

3. Results

3.1. Responses of the chronosequence soil physicochemical parameters to N and P additions

N additions significantly decreased soil pH from 4.77 to 4.41 in young soils, and from 4.04 to 3.72 in old soils (Fig. S2a). However, P additions did not significantly influence soil pH ($P > 0.05$). As the soil developed, more organic matter accumulated in the soil organic layer, thus total soil organic C in the old soils was about 2 times higher than in the young soils (Fig. S2b). Soil available N concentrations were about 7 times higher in old soils than those in young soils (Fig. S2c). P addition profoundly increased soil available P in young soils (Fig. S2d).

Compared with the young soils, soil total N significantly increased, but total P decreased in the old soils (Figs. S2e and f).

3.2. Effects of N and P additions on soil organism communities in two soil ages

Soil age, but not N and P additions, was related to the richness of soil organisms (Fig. 1). The richness of the bacteria, fungi, saprotroph, and AMF was higher (about 1.5, 1.5, 1.5 and 3.4 times, respectively) in young than old soils (Figs. 1 and S3). N additions significantly decreased the bacterial richness (Fig. 1a). Older soils were related to marginally decreased richness of protists (Fig. 1c). Moreover, soil bacterial biomass significantly increased with N additions in both soil ages, but decreased with P addition only in the old soils (Fig. S3d). However, soil fungal biomass profoundly increased with N additions in both soil ages (Fig. S3e). In addition, soil microbial activity (i.e., basal respiration, glucose- and lignin-induced respiration) had no responses to N and P additions in both soil ages (Fig. 1d-f).

Soil age, P addition, and their interactions profoundly impacted the composition of the bacterial and fungal communities, but N additions had only marginally significant effects on bacterial and fungal communities (Figs. S4a and b). The protists communities did vary with soil age (Fig. S4c). Nutrient addition and soil age had no effect on invertebrates (Fig. S4d). In addition, soil age had a greater effect (associated with soil acidification – as shown below) on soil microbial communities than N or P additions (Fig. S5). A total of 30% and 53% of the variation in bacterial and fungal communities was explained by soil age, respectively, which was much larger than the proportion explained by N and P additions (below 10% each, Figs. S5a and b).

3.3. System-level responses of belowground diversity to N and P additions and natural environmental gradients

The variance partitioning analysis also showed that the change of soil pH due to long-term pedogenesis was the main driver to explain the variance of bacteria and fungi community composition (Figs. S5 and S6). Similarly, the richness of bacteria, fungi and protists of the wet tropical ecosystems from four countries showed significantly positive relationships with soil pH by accounting for random effects of county/site (Fig. S7). Furthermore, the results of SEMs suggested that soil pH was the main control on the shifts in the richness of bacteria, fungi, and protists in the sites with N and P additions of three decades (Fig. 2a–c) and across environmental gradients of these wet tropical forests (Fig. 2d–f).

3.4. Relationships between belowground diversity and soil properties in Hawai'i experiment and environmental gradient soil survey

In Hawai'i wet tropical forests, the diversity of bacteria and fungi showed strong positive relationships with soil pH, but negative relationships with soil N and organic C (Fig. 3A). Overall, the diversity of dominant phyla and groups of bacteria and fungi displayed a similar pattern with that of bacteria and fungi communities in general (Fig. 3A). Moreover, in the environmental gradient soil survey, soil pH showed positive relationships with the diversity of protists, bacteria, fungi, the dominant phyla of bacteria (*Actinobacteria*, *Proteobracteria*, *Planctomycetes*, and *Chloroflexi*), and that of protists (*Lobosa* and *Conosa*) in wet tropical ecosystems from four continents (Fig. 3B). However, the main groups of invertebrates (*Annelida* and *Arthropoda*) showed strongly positive relationships with soil N and P, and organic C (Fig. 3B).

3.5. Belowground community composition responses to N and P additions

Soil age had consistent effects on the relative abundance of the dominant phyla of bacteria and fungi regardless of nutrient treatments (Figs. S8a and b). For example, the abundance of *Proteobacteria* was much higher, while the abundance of *Actinobacteria* was much lower in young than in old soils (Fig. S8a). For fungi, the abundance of

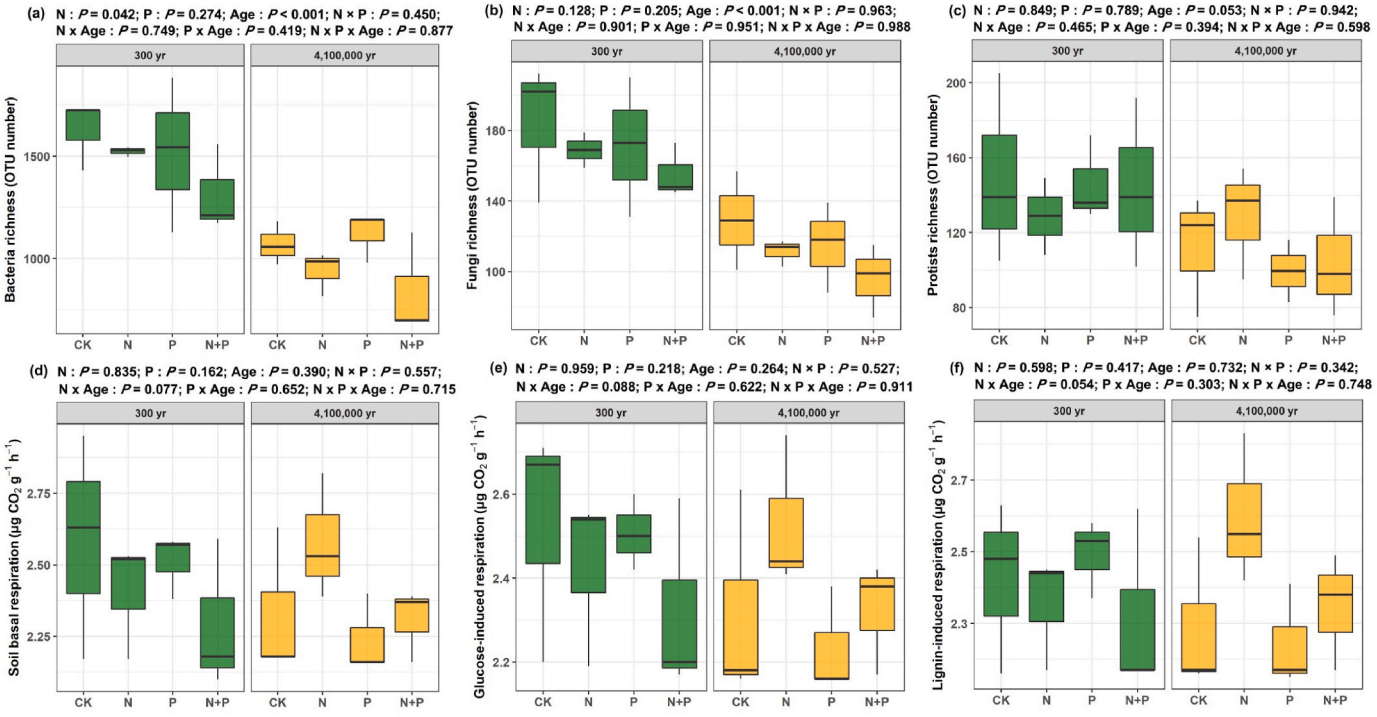

Fig. 1. Responses of the diversity of soil bacteria, fungi, protists, and soil basal and substrate-induced respiration (d–f) to N and P additions in two soil ages. The statistical results are three-way ANOVA for the effects of N addition, P addition and soil age. CK: control; N: N addition; P: P addition; N + P: Combination of N and P addition. The center line denotes the median value (50th percentile), while the box contains the 25th to 75th percentiles of the dataset. The black whiskers mark the 5th and 95th percentiles.

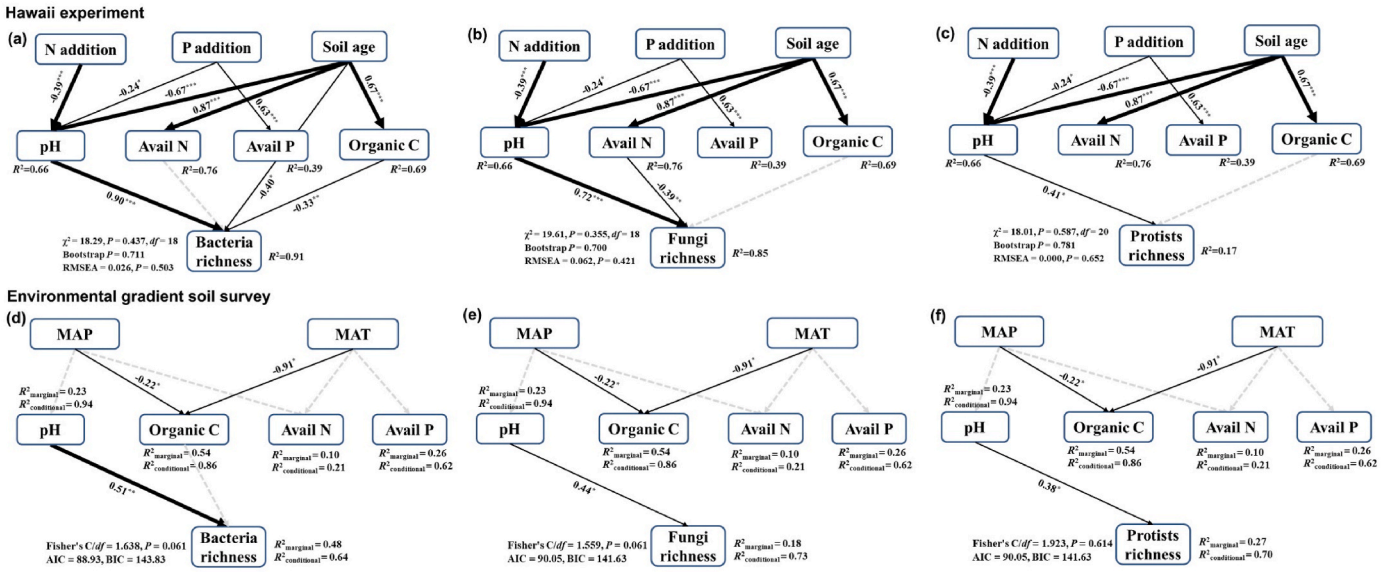

Fig. 2. Results of SEMs identify the direct and indirect relationships between the diversity of soil organisms and N and P additions, and soil substrate ages in the Hawai'i experiments (a–c) and tropical ecosystems in four continents (d–f). The diversity (OTU number, richness) of soil organisms includes bacteria, fungi, and protists. The variance explained by the model (R^2) of each parameter is given. $R^2_{conditional}$ denotes the proportion of variance explained by the included predictors without accounting for random effects of country/site. $R^2_{marginal}$ denotes the proportion of variance explained by the included predictors by accounting for random effects of country/site. Grey dashed arrows represent non-significant relationships in the models, which, however, improved the model fit when used ($P > 0.05$). Other non-significant SEM arrows were removed from the model to improve the model fit. Avail N, available N; Avail P, available P; MAP, mean annual precipitation; MAT, mean annual temperature; RMSEA, root mean square error of approximation. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Ascomycota was lower, but the abundance of *Basidiomycota* was much higher in young than in old soils (Fig. S8b). However, the effects of soil age on the dominant group of other soil organisms were variable among different N and P treatments (Figs. S8c–e). For instance, the abundance of *Cercozoa* protists under control and N and N + P treatments was higher in young soils compared to old soils, but was lower in young soils compared to old soils under the P addition treatment (Fig. S8c). For invertebrates, the abundances of *Annelida* and *Arthropoda* were lower in young than in old soils (Fig. S8d). For the main group of ARGs, the abundance of Beta-Lactamase was higher in young than in old soils under control and P addition conditions. In contrast, Beta-Lactamase abundance was lower in young than in old soils under N and N + P treatments (Fig. S8e).

3.6. Belowground opportunistic and sensitive responses to N and P additions

At the OTU level, the responses of the taxa of soil organisms to N and P additions depended on soil age (Fig. S9). P addition had larger effects (5.1% and 4.4%, respectively) on the relative abundance of opportunistic and sensitive bacterial taxa in old soils, but smaller effects in young soils (1.7% and 3.4%, respectively). Moreover, N additions increased the relative abundance of the opportunistic and sensitive bacterial taxa in young soils (4.8% and 3.4%, respectively), while it increased 2.9% and 4.6% in old soils (Fig. S9a). However, the effects of N and P additions together on the fungal taxa were larger than those of P addition alone (Fig. S9b). The proportion of the opportunistic and sensitive taxa of protists in old soils was larger than those in young soils (Fig. S9c). Similarly, 7.0% and 4.7% of the opportunistic and sensitive genes under N addition treatment, and 7.0% and 11.6% for P addition of ARGs genes were found in old soils, much larger than young soils (Fig. S9e). However, the taxa of invertebrates had small responses to N and P additions (Fig. S9d). At the species-level, the taxa responsive to N and P additions were dependent on soil ages (Tables S1–S4). For example, *Ciliophora* was only opportunistic to N additions in old soils, but *Nematoda* of invertebrates were sensitive to P addition in old soils (Table S3). Moreover, the relationships between bacteria, fungi richness and plant richness were not significant in wet tropical forests from four continents (Fig. S10).

4. Discussion

The environmental drivers of soil biodiversity are poorly understood in wet tropical forests – the lungs of the planet, and a fundamental refuge for biodiversity. Our work provides evidence that environmental filtering associated with acidification during long-term pedogenesis, and across environmental gradients, more than nutrient limitations, controls the biodiversity of bacteria, fungi and protists and that of important functional groups of soil organisms such as decomposers and arbuscular mycorrhizal fungi (common symbionts of the dominant tree species in these forests; e.g., *Metrosideros polymorpha* in Hawai'i). Similarly, long-term nutrient addition did not alter the activity of soil microbial communities measured as basal soil respiration, or respiration in response to glucose and lignin substrates. Nutrient additions did influence, however, the biomass of soil microbes, and the diversity of important functional genes (i.e., antibiotic resistance genes; Fig. S3). In general, our results suggest that environmental filtering by soil conditions more than plant-based effects (i.e., plant richness) controls soil biodiversity in these wet tropical ecosystems (Figs. 2 and S10), and thus, in contrast to what has been reported for plants (Ågren et al., 2012; Alvarez-Clare et al., 2013), P and N are not the main limiting factor associated with soil biodiversity in wet tropical forests. In addition, our results suggest that long-term N fertilization can actually have a negative impact on soil biodiversity by contributing to the further acidification of these soils.

The diversity of bacteria, fungi, saprotroph and arbuscular mycorrhizal fungi was considerably lower in old soils than in young soils (Figs. 1 and S3), which was predominantly related to the decreases in pH in these old and acid soils (Figs. 2, S6, S7). The results of soils from other wet tropical forests in four continents also supported our above findings in Hawai'i ecosystems (Figs. 2 and 3), which indicated that soil acidification may be the dominant driver of soil biodiversity under long-term pedogenesis and across environmental gradients in wet tropical

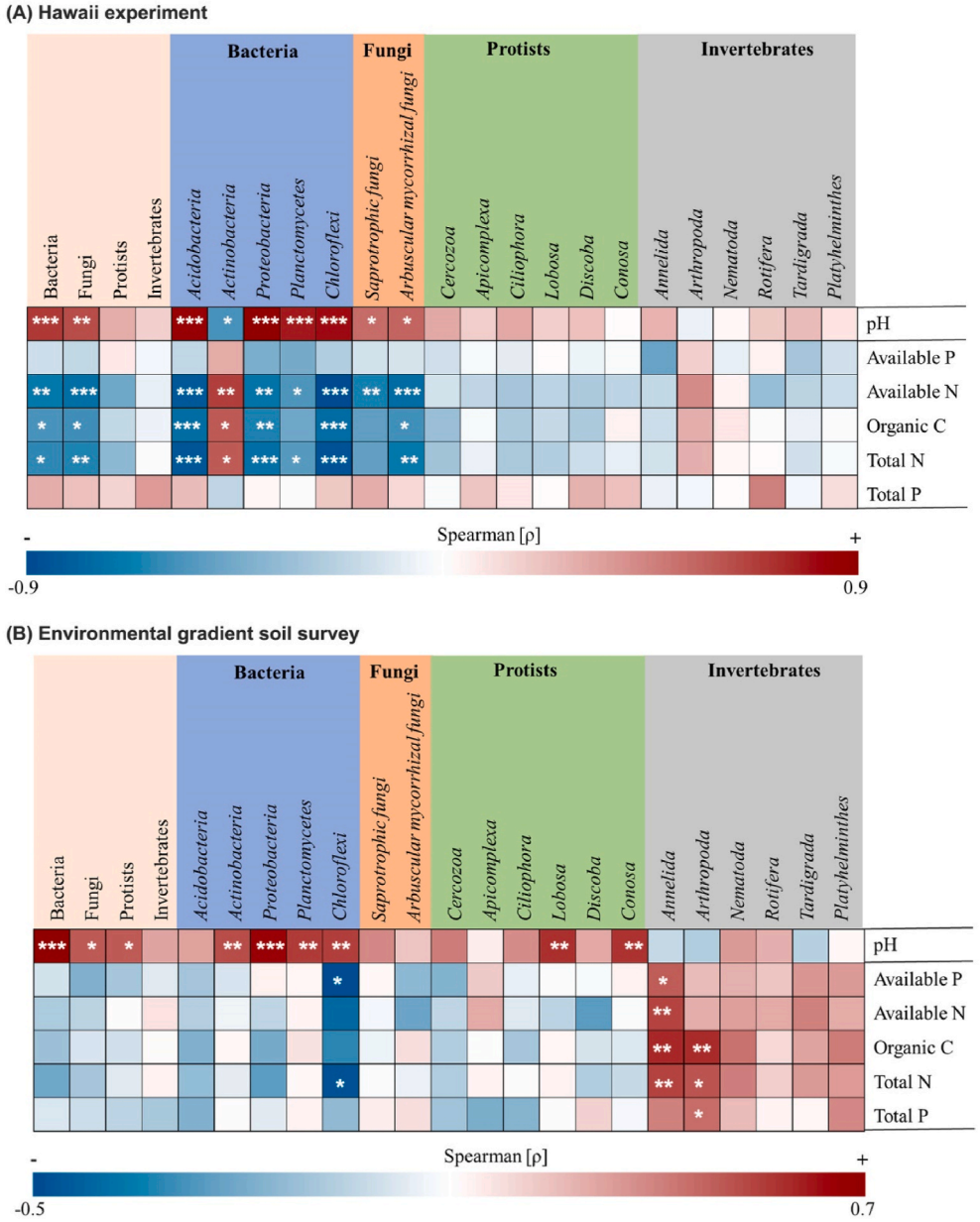

Fig. 3. Spearman correlations between the diversity of main groups of belowground organisms (including bacteria, fungi, protists, and invertebrates), and soil properties (including pH, available P and N, organic C, and total N and P) in the Hawai'i experiment (A) and the environmental gradient soil survey in tropical ecosystems in four continents (B). Significance relationships are indicated by asterisks; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

ecosystems. The small reductions in soil pH in already acid soils (here, below 5) (Fig. S2a), are known to have large negative impacts on soil biodiversity (e.g., Fierer and Jackson, 2006). We also found that soil age and N additions significantly decreased soil pH, which indirectly affected soil biodiversity, and these findings were in line with previous studies (Lauber et al., 2009; Yuan et al., 2016; Zhou et al., 2017; Alfaro et al., 2017; Ochoa-Hueso et al., 2018; Tripathi et al., 2018). Correspondingly, a recent global study showed that the reductions in pH typically observed along soil chronosequences were associated with the declines in the diversity of belowground organisms (Delgado-Baquerizo et al., 2019). Our results demonstrated that supplying limiting nutrient for plant productivity is insufficient to increase the diversity of soil organisms, especially in old and acid tropical forests. Indeed, the acidification effects associated with three decades of N additions resulted in strong reductions in soil biodiversity, and the negative effects were much more severe in old soils compared to young soils.

Nitrogen additions increased the biomass of bacteria and fungi, and the diversity of antibiotic resistance genes (Figs. S3d–f). The responses of microbial biomass were opposite to the diversity of belowground organisms, which supports the "paradox of enrichment" hypothesis (Rosenzweig, 1971; Bai et al., 2010). However, nutrient additions did not alter the activity of soil microbial communities in term of basal soil respiration, nor respiration in response to glucose and lignin substrates (Fig. 1d-f). These results indicated that the multiple aspects of soil microbial communities (such as diversity, biomass and activity) had contrasting responses to nutrient addition (negative, positive and null responses, respectively), in contrast to the previous findings in other terrestrial ecosystems (Treseder, 2008; Wang et al., 2018). A comprehensive understanding of the responses of soil microbial biomass and activity, and of reducing N fertilization application, is essential for the management and conservation of soil biodiversity in tropical ecosystems.

We also found that only a few rare taxa (i.e., opportunistic and sensitive species) had strong responses to N and P additions (Fig. S9). In

the relatively young soils with limited N availability, additions of this nutrient increased the proportion of opportunistic species in all bacterial taxa (Fig. S9a), which indicated that N was an important driver of these rare bacterial taxa in N-limited young soils of our study sites and previous research (Kurm et al., 2019). By contrast, P additions had stronger effects on the proportion of bacterial opportunistic and sensitive taxa in old soils than in young soils (Fig. S9a), suggesting P availability was the main controlling factor of the soil microbial community in P-limited old soils (Samaddar et al., 2019; Oliverio et al., 2020). These results demonstrated that soil background nutrient concentrations, as dependent on long-term soil development, determine the effects of N and P additions on belowground organisms at the species level (Vitousek et al., 2010; Zhang et al., 2014; Wardle et al., 2016; Ochoa-Hueso et al., 2018). Thus, different measures are required to maintain soil biodiversity and sustainable ecosystem functioning of wet tropical forests based on the local conditions (such as which nutrients are most limiting). While there was no significant relationship between plant richness and bacterial or fungal richness, protist richness had a small but significant relationship with plant richness (Fig. S10c). Our results were supported by the findings of previous studies, detecting the strong links between protists and plant communities (Ceja-Navarro et al., 2021; Guo et al., 2021). Our study indicated that the biodiversity loss, especially plant diversity under global change, might have large negative impacts on protists diversity, and further alter ecosystem structures and functions (Cardinale et al., 2012).

In conclusion, our study revealed that the environmental filtering via soils and not plant-based richness drives the biodiversity of multiple belowground organisms during a long-term pedogenesis conducted with three decades of N and P additions and across environmental gradients of wet tropical ecosystems. The long-term pedogenesis led to soil acidification attributed to a contrasting decrease in soil pH, from young to old soils. Likewise, the diversity of bacterial and fungal communities was lower in old compared to young soils. Nutrient enrichment negatively affected the biodiversity of most belowground organisms studied here. For instance, N additions decreased the bacterial and fungal richness in both soil types. The negative responses of soil microbial diversity to nutrient additions were attributable to the acidification effects and decreased soil pH with N fertilization in these ecosystems. The detrimental effects of N additions on the biodiversity of belowground organisms were much larger in old soils where P supply was limiting. These wet tropical forests with severely acid soils are commonly limited by low N availability in young soils, while old soils have low P availability. However, the negative effects of soil acidification induced by N addition we observed were much stronger than the potential positive effects of limited nutrient supply on soil biodiversity. Nevertheless, some results varied in important ways from other terrestrial ecosystem types, and our sites cannot capture the large heterogeneity across tropical forests. Further study is needed to advance our understanding of the mechansisms controlling belowground communities in wet tropical forests. Taken together, our results demonstrate that the N additions, resulting in acidification processes do not help to maintain biodiversity of belowground organisms, even in highly-weathered wet tropical forest soils with low P availability. Instead, our data suggest that soil acidification is the dominant driver of the loss of soil biodiversity after artificial nutrient addition.

Authors' contributions

M.D-B., H.Y.C. and P.M.V. developed the ideas in this study. M.D-B., S.C.R., B.S., A.R.B., J.P.V., A.M., G.F. P–B., A.L.T., P.T., J-Z. H., H–W. H. and K.P. conducted the experiments and collected the data. H.Y.C analyzed the data and wrote the initial draft of the manuscript with the help of M.D-B. S.C.R., W.S., B.S., A.R.B., J.P.V., A.M., G.F. P–B., A.L.T., P.T., J-Z. H., H–W. H. and K.P. provided substantial feedback and comments to revise the manuscript.

Availability of data and materials

The database used for this paper is available from Figshare (https://doi.org/10.6084/m9.figshare.16661269).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108571.

References

Achard, F., Eva, H.D., Stibig, H.-J., Mayaux, P., Mayaux, P., Gallego, J., Richards, T., Malingreau, J.-P., 2002. Determination of deforestation rates of the world's humid tropical forests. Science 297 (5583), 999–1002.

Ågren, G.I., Wetterstedt, J.Å.M., Billberger, M.F.K., 2012. Nutrient limitation on terrestrial plant growth – modeling the interaction between nitrogen and phosphorus. New Phytologist 194 (4), 953–960.

Alfaro, F.D., Manzano, M., Marquet, P.A., Gaxiola, A., 2017. Microbial communities in soil chronosequences with distinct parent material: the effect of soil pH and litter quality. Journal of Ecology 105, 1709–1722.

Alvarez-Clare, S., Mack, M.C., Brooks, M., 2013. A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. Ecology 94 (7), 1540–1551.

Anderson, J.M., Ingram, J.S.I., 1993. Tropical Soil Biology and Fertility: A Handbook of Methods, second ed. CABI, Wallingford UK.

Bai, Y., Wu, J., Clark, C.M., Naeem, S., Pan, Q., Huang, J., Zhang, L., Han, X.-G., 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Global Change Biology 16, 358–372.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511.

Bakker, M.R., Brunner, I., Ashwood, F., Bjarnadottir, B., Bolger, T., Børja, I., Carnol, M., Cudlin, P., Dalsgaard, L., Erktan, A., Godbold, D., Kraigher, H., Meier, I.C., Merino-Martín, L., Motiejünaitė, J., Mrak, T., Oddsdóttir, E.S., Ostonen, I., Pennanen, T.L., Püttsepp, Ü., Suz, L.M., Vanguelova, E.I., Vesterdal, L., Soudzilovskaia, N.A., 2019. Belowground biodiversity relates positively to ecosystem services of European forests. Frontiers in Foreats and Global Change 2, 1–23.

Bates, D., Mäechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., 2017. lme4: linear mixed-effects models using Eigen and S4. R Package Version 1, 1–13.

Beck, H., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. Scientific Data 5, 180214.

Buyer, J.S., Sasser, M., 2012. High throughput phospholipid fatty acid analysis of soils. Applied Soil Ecology 61, 127–130.

Cameron, E.K., Martins, I.S., Lavelle, P., Mathieu, J., Tedersoo, L., Bahram, M., Gottschall, F., Guerra, C.A., Hines, J., Patoine, G., Siebert, J., Winter, M., Cesarz, S., Ferlian, O., Kreft, H., Lovejoy, T.E., Montanarella, L., Orgiazzi, A., Pereira, H.M.,

Phillips, H.R.P., Settele, J., Wall, D.H., Eisenhauer, N., 2019. Global mismatches in aboveground and belowground biodiversity. Conservation Biology 33 (5), 1187–1192.
Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6, 1621–1624.
Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67.
Ceja-Navarro, J.A., Wang, Y., Ning, D., Arellano, A., Ramanculova, L., Yuan, M., Byer, A., Craven, K.D., Saha, M.C., Brodie, E.L., Pett-Ridge, J., Firestone, M.K., 2021. Protist diversity and community complexity in the rhizosphere of switchgrass are dynamic as plants develop. Microbiome 9, 96.
Chadwick, O.A., Derry, L.A.P., Vitousek, M., Huebert, B.J., Hedin, L.O., 1999. Changing sources of nutrients during four million years of ecosystem development. Nature 397, 491–497.
Clark, C., Tilman, D., 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451, 712–715.
Clausing, S., Likulunga, L.E., Janz, D., Feng, H.Y., Schneider, D., Daniel, R., Krüger, J., Lang, F., Polle, A., 2020. Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests. bioRxiv 12 (29), 424645.
Cui, H., Sun, W., Delgado-Baquerizo, M., Song, W., Ma, J.-Y., Wang, K., Ling, X., 2020. The effects of mowing and multi-level N fertilization on soil bacterial and fungal communities in a semiarid grassland are year-dependent. Soil Biology and Biochemistry 151, 108040.
Delgado-Baquerizo, M., Maestre, F., Gallardo, A., Bowker, M.A., Wallenstein, M.D., Quero, J.L., Ochoa, V., Gozalo, B., García-Gómez, M., Soliveres, S., García-Palacios, P., Berdugo, M., Valencia, E., Escolar, C., Arredondo, T., Barraza-Zepeda, C., Bran, D., Carreira, J.A., Chaieb, M., Conceição, A.A., Derak, M., Eldridge, D.J., Escudero, A., Espinosa, C.I., Gaitán, J., Gatica, M.G., Gómez-González, S., Guzman, E., Gutiérrez, J.R., Florentino, A., Hepper, E., Hernández, R. M., Huber-Sannwald, E., Jankju, M., Liu, J., Mau, R.L., Miriti, M., Monerris, J., Naseri, K., Noumi, Z., Polo, V., Prina, A., Pucheta, E., Ramírez, E., Ramírez-Collantes, D.A., Romão, R., Tighe, M., Torres, D., Torres-Díaz, C., Ungar, E.D., Val, J., Wamiti, W., Wang, D., Zaady, E., 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502, 672–676.
Delgado-Baquerizo, M., Eldridge, D.J., Ochoa, V., Gozalo, B., Singh, B.K., 2017a. Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. Ecology Letters 20, 1295–1305.
Delgado-Baquerizo, M., Reich, P.B., Khachane, A.N., Campbell, C.D., Thomas, N., Freitag, T.E., Al-Soud, W.A., Sørensen, S., Bardgett, R.D., Singh, B.K., 2017b. It is elemental: soil nutrient stoichiometry drives bacterial diversity. Environmental Microbiology 19, 1176–1188.
Delgado-Baquerizo, M., Trivedi, P., Trivedi, C., Eldridge, D.J., Reich, P.B., Jeffries, T.C., Singh, B.K., 2017c. Microbial richness and composition independently drive soil multifunctionality. Functional Ecology 31, 2330–2343.
Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D. J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325.
Delgado-Baquerizo, M., Bardgett, R.D., Vitousek, P.M., Maestre, F.T., Williams, M.A., Eldridge, D.J., Lambers, H., Neuhauser, S., Gallardo, A., García-Velázquez, L., Sala, O.E., Currier, C.M., Cutler, N.A., Hart, S.C., Hayes, P.E., Hseu, Z.-Y., Kirchmair, M., Peña-Ramírez, V.M., Pérez, C.A., Reed, S.C., Santos, F., Siebe, C., Sullivan, B.W., Weber-Grullon, L., Fierer, N., 2019. Changes in belowground biodiversity during ecosystem development. Proceedings of the National Academy of Sciences of the United States of America 116 (14), 6891–6896.
Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D., Bastida, F., Berhe, A.A., Cutler, N.A., Gallardo, A., García-Velázquez, L., Hart, S.C., Hayes, P.E., He, J.-Z., Hseu, Z.-Y., Hu, H.-W., Kirchmair, M., Neuhauser, S., Pérez, A. C., Reed, S.C., Santos, F., Sullivan, B.W., Trivedi, P., Wang, J.-T., Weber-Grullon, L., Williams, M.A., Singh, B.K., 2020a. Multiple elements of soil biodiversity drive ecosystem functions across biomes. Nature Ecology and Evolution 4, 210–220.
Delgado-Baquerizo, M., Reich, P.B., Bardgett, R.D., Eldridge, D.J., Lambers, H., Wardle, D.A., Reed, S.C., Plaza, C., Png, G.K., Neuhauser, S., Berhe, A.A., Hart, S.C., Hu, H.-W., He, J.-Z., Bastida, F., Abades, S., Alfaro, F.D., Cutler, N.A., Gallardo, A., García-Velázquez, L., Hayes, P.E., Hseu, Z.-Y., Pérez, C.A., Santos, F., Siebe, C., Trivedi, P., Sullivan, B.W., Weber-Grullon, L., Williams, M.A., Fierer, N., 2020b. The influence of soil age on ecosystem structure and function across biomes. Nature Communications 11, 4721.
Delgado-Baquerizo, M., Guerra, C.A., Cano-Díaz, C., Egidi, E., Wang, J.-T., Eisenhauer, N., Singh, B.K., Maestre, F.T., 2020c. The proportion of soil -bornepathogens increases with warming at the global scale. Nature Climate Change 10, 550–554.
Ding, J., Zhu, D., Chen, Q.-L., Zheng, F., Wang, H.-T., Zhu, Y.-G., 2019. Effects of long-term fertilization on the associated microbiota of soil collembolan. Soil Biology and Biochemistry 130, 141–149.
Edgar, R.G., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996–998.
Edgar, R.C., 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv, 081257.
Edwards, D.P., Tobias, J.A., Sheil, D., Meijaard, E., Laurance, W.F., 2014. Maintaining ecosystem function and services in logged tropical forests. Trends in Ecology & Evolution 29 (9), 511–520.
Evans, S.E., Wallenstein, M.D., 2014. Climate change alters ecological strategies of soil bacteria. Ecology Letters 7, 155–164.
Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15, 579–590.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626–631.
Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65.
Grace, J.B., 2006. Structural Equation Modeling and Natural Systems. Cambridge University Press, Cambridge UK.
Guerra, C.A., Heintz-Buschart, A., Sikorski, J., Chatzinotas, A., Guerrero-Ramirez, N., Cesarz, S., Beaumelle, L., Rillig, M.C., Maestre, F.T., Delgado-Baquerizo, M., Buscot, F., Overmann, J., Patoine, G., Phillips, H.R.P., Winter, M., Wubet, T., Küsel, K., Bardgett, R.D., Cameron, E.K., Cowan, D., Grebenc, T., Marín, C., Orgiazzi, A., Singh, B.K., Wall, D.H., Eisenhauer, N., 2020. Blind spots in global soil biodiversity and ecosystem function research. Nature Communications 11 (1), 3870.
Guo, S., Xiong, W., Hang, X., Gao, Z., Jiao, Z., Liu, H., Mo, Y., Zhang, N., Kowalchuk, G. A., Li, R., Shen, Q., Geisen, S., 2021. Protists as main indicators and determinants of plant performance. Microbiome 9, 64.
Hobbie, S.E., Vitousek, P.M., 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81 (7), 1867–1877.
Hu, H.-W., Wang, J.-T., Singh, B.K., Liu, Y.-R., Chen, Y.-L., Zhang, Y.-J., He, J.-Z., 2018. Diversity of herbaceous plants and bacterial communities regulates soil resistome across forest biomes. Environmental Microbiology 20, 3186–3200.
Johnston, E.R., Kim, M., Hatt, J.K., Phillips, J.R., Yao, Q., Song, Y., Hazen, T.C., Mayes, M.A., Konstantinidis, K.T., 2019. Phosphate addition increases tropical forest soil respiration primarily by deconstraining microbial population growth. Soil Biology and Biochemistry 130, 43–54.
Kitayama, K., Mueller-Dombois, D., 1995. Vegetation changes during long-term soil development in the Hawaiian montane rainforest zone. Vegetatio 120, 1–20.
Kurm, V., Geisen, S., Gera Hol, W.H., 2019. A low proportion of rare bacterial taxa responds to abiotic changes compared with dominant taxa. Environmental Microbiology 21 (2), 750–758.
Laliberté, E., Grace, J.B., Huston, M.A., Lambers, H., Teste, F., Turner, B.L., Wardle, D.A., 2013. How does pedogenesis drive plant diversity? Trends in Ecology & Evolution 28 (6), 331–340.
Laliberté, E., Zemunik, G., Turner, B.L., 2014. Environmental filtering explains variation in plant diversity along resource gradients. Science 345, 1602–1605.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Soil pH as a predictor of soil bacterial community structure at the continental scale: a pyrosequencing-based assessment. Applied and Environmental Microbiology 75, 5111–5120.
Leff, J.W., Jones, S.E., Prober, S.M., Barberan, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., Pierre, K.L., Risch, A. C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences of the United States of America 112, 10967–10972.
Lefcheck, J.S., 2016. PiecewiseSEM: piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods in Ecology and Evolution 7, 573–579.
Liu, J., Li, X., Ma, Q., Zhang, X., Chen, Y., Isbell, F., Wang, D., 2019. Nitrogen addition reduced ecosystem stability regardless of its impacts on plant diversity. Journal of Ecology 107 (5), 2427–2435.
Liu, S., Wang, H., Tian, P., Yao, X., Sun, H., Wang, Q., Delgado-Baquerizo, M., 2020. Decoupled diversity patterns in bacteria and fungi across continental forest ecosystems. Soil Biology and Biochemistry 144, 107763.
Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., García-Gómez, M., Bowker, M.A., Soliveres, S., Escolar, C., García-Palacios, P., Berdugo, M., Valencia, E., Gozalo, B., Gallardo, A., Aguilera, L., Arredondo, T., Blones, J., Boeken, B., Bran, D., Conceição, A.A., Cabrera, O., Chaieb, M., Derak, M., Eldridge, D.J., Espinosa, C.I., Florentino, A., Gaitán, J., Gatica, M.G., Ghiloufi, W., Gómez-González, S., Gutiérrez, J.R., Hernández, R.M., Huang, X., Huber-Sannwald, E., Jankju, M., Miriti, M., Monerris, J., Mau, R.L., Morici, E., Naseri, K., Ospina, A., Polo, V., Prina, A., Pucheta, E., Ramírez-Collantes, D.A., Romão, R., Tighe, M., Torres-Díaz, C., Val, J., Veiga, J.P., Wang, D., Zaady, E., 2012. Plant species richness and ecosystem multifunctionality in global drylands. Science 335, 214–218.
Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., Eldridge, D.J., Ochoa, V., Gozalo, B., Quero, J.L., García-Gómez, M., Gallardo, A., Ulrich, W., Bowker, M.A., Arredondo, T., Barraza-Zepeda, C., Bran, D., Florentino, A., Gaitán, J., Gutiérrez, J. R., Huber-Sannwald, E., Jankju, M., Mau, R.L., Miriti, M., Naseri, K., Ospina, A., Stavi, I., Wang, D., Woods, N.N., Yuan, X., Zaady, E., Singh, B.K., 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceedings of the National Academy of Sciences 112, 15684–15689.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248.
Ochoa-Hueso, R., Eldridge, D.J., Delgado-Baquerizo, M., Soliveres, S., Bowker, M.A., Gross, N., Bagousse-Pinguet, Y.L., Quero, J.L., García-Gómez, M., Valencia, E., Arredondo, T., Beinticinco, L., Bran, D., Cea, A., Coaguila, D., Dougill, A.J., Espinosa, C.I., Gaitán, J., Guuroh, R.T., Guzman, E., Gutiérrez, J.R., Hernández, R. M., Huber-Sannwald, E., Jeffries, T., Linstädter, A., Mau, R.L., Monerris, J., Prina, A., Pucheta, E., Stavi, I., Thomas, A.D., Zaady, E., Singh, B.K., Maestre, F.T., 2018. Soil fungal abundance and plant functional traits drive fertile island formation in global drylands. Journal of Ecology 106, 242–253.
Oliverio, A.M., Bissett, A., McGuire, K., Saltonstall, K., Turner, B.L., Fierer, N., 2020. The role of phosphorus limitation in shaping soil bacterial communities and their metabolic capabilities. mBio 11, e01718–e01720.
Ramirez, K.S., Leff, J.W., Barberán, A., Bates, S.T., Betley, J., Crowther, T.W., Kelly, E.F., Oldfield, E.E., Shaw, E.A., Steenbock, C., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Proc R Soc B 281, 20141988.
Reed, S.C., Vitousek, P.M., Cleveland, C.C., 2011. Are patterns in nutrient limitation belowground consistent with those aboveground: results from a 4 million year chronosequence. Biogeochemistry 106 (3), 323–336.
Rosenzweig, M.L., 1971. Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. Science 171 (3969), 385–387.
Samaddar, S., Chatterjee, P., Truu, J., Anandham, R., Kim, S., Sa, T., 2019. Long-term phosphorus limitation changes the bacterial community structure and functioning in paddy soils. Applied Soil Ecology 134, 111–115.
Soliveres, S., van der Plas, F., Manning, P., Prati, D., Gossner, M.M., Renner, S.C., Alt, F., Arndt, H., Baumgartner, V., Binkenstein, J., Birkhofer, K., Blaser, S., Blüthgen, N., Boch, S., Böhm, S., Börschig, C., Buscot, F., Diekötter, T., Heinze, J., Hölzel, N., Jung, K., Klaus, V.H., Kleinebecker, T., Klemmer, S., Krauss, J., Lange, M., Morris, E. K., Müller, J., Oelmann, Y., Overmann, J., Pašalić, E., Rillig, M.C., Schaefer, H.M., Schloter, M., Schmitt, B., Schöning, I., Schrumpf, M., Sikorski, J., Socher, S.A., Solly, E.F., Sonnemann, I., Sorkau, E., Steckel, J., Steffan-Dewenter, I., Stempfhuber, B., Tschapka, M., Türke, M., Venter, P.C., Weiner, C.N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, W., Wolters, V., Wubet, T., Wurst, S., Fischer, M., Allan, E., 2016. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. Nature 536, 456–459.
Souza, R.C., Souza, F.C., Maia, V.A., Aguiar-Campos, N., Coelho, P.A., Farrapo, C.L., Santos, A.B.M., Araújo, F.C., Gianasi, F.M., Paula, G.G.P., Morel, J.D., Fagundes, N.C. A., Garcia, P.O., Santos, P.F., Silva, W.B., Fontes, M.A.L., Santos, R.M., 2021. Tropical forests structure and diversity: a comparison of methodological choices. Methods Ecol. Evol. https://doi.org/10.1111/2041-210X.13670.
Tarlera, S., Jangid, K., Ivester, A.H., Whitman, W.B., Williams, M.A., 2008. Microbial community succession and bacterial diversity in soils during 77,000 years of ecosystem development. FEMS Microbiology Ecology 64, 129–140.
Turner, B.L., Brenes-Arguedas, T., Condit, R., 2018. Pervasive phosphorus limitation of tree species but not communities in tropical forests. Nature 555 (7696), 367–370.
Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. The ISME Journal 12, 1072–1083.
Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11, 1111–1120.
Vitousek, P.M., Farrington, H., 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37, 63–75.
Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms implications and nitrogen-phosphorus interactions. Ecological Applications 20, 5–15.
Wardle, D.A., Walker, L.R., Bardgett, R.D., 2004. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305 (5683), 509–513.
Wardle, D.A., Bardgett, R.D., Walker, L.R., Peltzer, D.A., Lagerström, A., 2008. The response of plant diversity to ecosystem retrogression: evidence from contrasting long-term chronosequences. Oikos 117, 93–103.
Wardle, D.A., Wardle, R.D., Walker, L.R., Bonner, K.I., 2009. Among- and within-species variation in plant litter decomposition in contrasting long-term chronosequences. Functional Ecology 23, 442–453.
Wardle, D.A., Jonsson, M., Mayor, J.R., Metcalfe, D.B., 2016. Above-ground and below-ground responses to long-term nutrient addition across a retrogressive chronosequence. Journal of Ecology 104 (2), 545–560.
Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of chronosequences in studies of ecological succession and soil development. Journal of Ecology 98, 725–736.
Wang, C., Liu, D., Bai, E., 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biology and Biochemistry 120, 126–133.
Wagg, C., Bender, F.S., Widmer, F., van der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America 111, 5266–5270.
Wright, T.C., Helz, R.T., 1987. In: Decker, R.W., et al. (Eds.), Volcanism in Hawaii, 1350. US Geol Surv Prof Paper, pp. 625–640.
Yuan, X., Knelman, J.E., Gasarch, E., Wang, D., Nemergut, D.R., Seastedt, T.R., 2016. Plant community and soil chemistry responses to long-term nitrogen inputs drive changes in alpine bacterial communities. Ecology 97, 1543–1554.
Zhang, X., Wei, H., Chen, Q., Han, X., 2014. The counteractive effects of nitrogen addition and watering on soil bacterial communities in a steppe ecosystem. Soil Biology and Biochemistry 72, 26–34.
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. Scientific Reports 7, 3267.
Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nature Communications 11, 3072.
Zhu, D., Delgado-Baquerizo, M., Ding, J., Gillings, M.R., Zhu, Y.-G., 2021. Trophic level drives the host microbiome of soil invertebrates at a continental scale. Microbiome 9, 189.