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Predicting the responsiveness of soil biodiversity to deforestation: a cross-biome study

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Abstract

The consequences of deforestation for aboveground biodiversity have been a scientific and political concern for decades. In contrast, despite being a dominant component of biodiversity that is essential to the functioning of ecosystems, the responses of belowground biodiversity to forest removal have received less attention. Single-site studies suggest that soil microbes can be highly responsive to forest removal, but responses are highly variable, with negligible effects in some regions. Using high throughput sequencing, we characterize the effects of deforestation on microbial communities across multiple biomes and explore what determines the vulnerability of microbial communities to this vegetative change. We reveal consistent directional trends in the microbial community response, yet the magnitude of this vegetation effect varied between sites, and was explained strongly by soil texture. In sandy sites, the difference in vegetation type caused shifts in a suite of edaphic characteristics, driving substantial differences in microbial community composition. In contrast, fine-textured soil buffered microbes against these effects and there were minimal differences between communities in forest and grassland soil. These microbial community changes were associated with distinct changes in the microbial catabolic profile, placing community changes in an ecosystem functioning context. The universal nature of these patterns allows us to predict where deforestation will have the strongest effects on soil biodiversity, and how these effects could be mitigated.

Keywords: Soil Biodiversity, Deforestation, Microbial community, Ecosystem functioning, Metagenomic sequencing

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Introduction

Forest ecosystems support a substantial proportion of global biodiversity and represent a carbon (C) sink of approximately 2.4 Pg per year (Pan *et al.*, 2011). Both features are threatened by the conversion of natural forests to grasslands for timber or agricultural practices. The loss of soil carbon resulting from this land-use change accounts for 12% of total anthropogenic contributions to current climate change (Van der Werf *et al.*, 2009), and associated species extinctions are a major component of global biodiversity loss in recent decades (Sala, 2000). As the dominant drivers of biogeochemical cycles and a prominent component of biodiversity in terrestrial ecosystems (Mora *et al.*, 2011), soil microbes (predominantly fungi, bacteria and archaea) intrinsically link these two features of forest ecosystems(Van der Heijden *et al.*, 2008). Understanding the mechanisms that control, and the functional consequences of, microbial community responses to forest removal, therefore, remains among the biggest challenges for basic and applied ecology.

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Traditionally, the highly complex and opaque nature of the soil environment has stymied efforts to identify the effects of land-use change on microbial communities. The majority of studies have documented changes in traditional microbial parameters, such as biomass or activity, and often reveal reductions in both following deforestation(Dinesh *et al.*, 2003; Holden & Treseder, 2013). However, the advent of next-generation sequencing has provided unique insight into soil microbial diversity and the processes structuring communities on a global scale (Fierer *et al.*, 2012). Two recent studies conducted in the Brazilian rainforest suggest that, in stark contrast with aboveground plants and animals, bacterial diversity can increase following deforestation (Da C Jesus *et al.*, 2009; Rodrigues *et al.*, 2013). These observations were correlated with reductions in soil C and nitrogen (N) concentrations, suggesting that the loss of organic matter following forest removal may drive microbial community responses. On a global-scale, however, the effects of forest harvesting on soil C and N stocks are more idiosyncratic (Johnson & Curtis, 2001), and there are negligible effects on microbial biomass in several regions (Holden & Treseder, 2013). No study, to date, has explored this idiosyncracy, by

estimating community responses across multiple biome types. As a result, we have a limited understanding of the general trends that characterize microbial community responses to deforestation, or the mechanisms governing the susceptibility of soil biodiversity to disturbance across broad spatial scales. Thus, we are unable to anticipate the vulnerability of different regions to such a land-use change.

The limited representation of fungi, the dominant decomposing agents in woodland ecosystems (Hättenschwiler *et al*., 2005), in community-scale analyses represents another substantial gap in our understanding of soil biodiversity responses to deforestation, even within single sites. Theoretical models predict that via the formation of long-lived networks of hyphae, fungal communities are more resistant (but not resilient) than bacteria to land-use change (De Vries *et al*., 2012a). However, since previous empirical studies have focused on fungal biomass, fruiting bodies or specific groups of mycorrhizal fungi (Aldrich-Wolfe, 2007; Öpik *et al*., 2013), the susceptibility of entire fungal communities to forest removal remains unresolved. A continental-scale, cross-biome approach assessing fungi, bacteria and their functional potential is essential to identify the unifying principles governing the susceptibility of soil biodiversity to forest removal.

The lack of empirical studies exploring soil microbial responses to deforestation across multiple biomes reflects the inherent challenges in identifying appropriate/comparable sites, and characterizing diverse microbial communities at a broad spatial scale. We take advantage of a unique set of well-maintained, long-term experimental research sites across the USA and use high-throughput sequencing to explore community-scale responses across tropical, temperate, and boreal biomes. Each paired site consisted of a natural forest adjacent to a previously forested old-field grassland. To incorporate potential legacy effects of forests on soil microbial communities, we included sites that varied substantially in the time since forest removal (ranging from <10 to >100 years). These also included two sites containing a naturally formed grassland to identify if general taxonomic differences are representative of the vegetation type or simply the disturbance of forest removal. We explore (i) the general trends characterizing the microbial community response to deforestation and (ii) the site-specific characteristics governing microbial susceptibility to vegetation change at the continental scale. We predicted that: (i) there will be consistent microbial community responses to deforestation (Rodrigues *et al*., 2013), but the magnitude of these effects will vary between sites, increasing linearly with time since forest conversion (As seen for changes in biomass following deforestation; Holden & Treseder, 2013) and (ii) the rate of the community response will be related to climatic conditions, particularly temperature and moisture conditions which represent key controls on the activity of microbial communities (Bryant *et al*., 2008; Crowther & Bradford, 2013). We further tested the prediction that microbial community changes following deforestation should lead to proportional changes in the functional capabilities of belowground communities on a continent-scale.

Materials and methods

Soil collection and sampling design

Soils were collected from 11 regions ranging from Hawaii to Northern Alaska, using a sampling design intended to maximize variation in climatic conditions and biome types (Table S1; Figure S1). Sites included 10 Long Term Ecological Research stations [Andrews Experimental Forest, Oregon 44.21 °N, −122.26 °E (AND), Bonanza Creek, Alaska 64.85 °N, −147.84 °E (BNZ), Cedar Creek Ecosystem Science Reserve, Minnesota 45.40 °N, −93.20 °E (CDR), Coweeta LTER, North Carolina 35 °N, −83.5 °E (CWT), Hubbard Brook Experimental Forest, New Hampshire 43.94 °N, −71.75 °E (HBR), Harvard Forest, Massachusetts 42.53 °N, −72.19 °E (HFR), Kellogg Biological Station, Michigan, 42.4 °N, −85.4 °E (KBS), Konza Prairie Biological Station, Kansas 39.09 °N, −96.57 °E (KNZ), Luquillo LTER, Puerto Rico 18.3 °N, −65.8 °E (LUQ) and Niwot Ridge LTER, Colorado 39.99 °N, −105.37 °E (NWT)], and the Hawaii Experimental Tropical Forest, Institute of Pacific Islands Forestry, Hawaii 19.81 °N, −155.26 °E (HAW). Each paired site included a natural forest and adjacent grassland, manipulated by low-intensity (annual mowing) management or unmanaged. To incorporate potential legacy effects of forests on soil microbial communities, our study design included sites that varied substantially in the time since vegetation change (Table S1).

Microbial community attributes can vary seasonally and annually (Baldrian *et al*., 2012; Lauber *et al*., 2013). We attempted to capture this temporal variation in microbial communities and identify the upper and lower bounds of the response to deforestation. To capture the maximum possible temporal variation in microbial community attributes, three replicate soil samples (0–10 cm depth) were collected from each sampling site over three years, with each annual sampling date falling in a different season (spring, summer, and Fall respectively). Each soil sample was homogenized and divided up for different community/functioning analyses and stored at −80 °C, prior to use. We also included two sites containing a naturally formed grassland to identify if general taxonomic differences are representative of the vegetation type or simply the disturbance of forest removal. Soils were sieved to 2 mm, screened to remove remaining root and litter fragments, and then homogenized to provide a total of 66 soil samples (11 regions × 2 vegetation types × 3 time points).

Soils were analyzed for pH by mixing water to soil in a 1 : 1 volumetric ratio and gravimetric moisture was determined by

oven drying to constant mass at 105 °C. Total soil C and N content was determined on an elemental analyzer (LECO, St Joseph, MI, USA). Sand, silt, and clay contents were measured using a simplified version of the hydrometer method following Bouyoucos (1927), and the remaining soil was used for microbial community analysis.

Phospholipid fatty acid (PLFA) analysis

We assessed active microbial biomass using PLFA analysis. This methodology followed that of Findlay & Dobbs (1993). Specifically, samples were homogenized and 5 g moist soil was extracted in a single phase, phosphate-buffered dichloromethane solution to remove PLFAs. These lipids were further separated by silicic acid chromatography, and phospholipids were derivatized in an alkaline solution to form fatty acid methyl esters (FAMEs). FAME purification was performed with C18 reverse plasma chromatography. These were then separated and quantified by capillary gas chromatography with a flame ionization detector (Shimadzu 2014 GC, Shimadzu Corp., Tokyo, Japan) equipped with a Restek Rtx-1 column (Restek Corp., Bellefonte, PA, USA). FAME peaks were identified and concentrations calculated based on a Supelco 37 Component FAME mix (Sigma-Aldrich Co., St. Louis, MO, USA) run as a standard every third sample. Bacterial biomass was calculated as the sum of the following fatty acids: i14 : 0, i15 : 0, a15 : 0, i16 : 0, i17 : 0, a17 : 0, 16 : 1n9, 16 : 1n7, cy17 : 0, 18 : 1n7, 18 : 1n5, cy19 : 0, 14 : 0, 15 : 0, 17 : 0, 18 : 0, 10Me16, 10Me17, 10Me18, i17 : 1n7 (nmol g^{-1} soil). Fungal biomass was the sum of 18 : 2n6 and 18 : 1n9 (nmol g^{-1} soil).

High-throughput DNA sequencing

We used a barcoded high-throughput sequencing approach similar to that described previously (Caporaso *et al*., 2012) to assess the diversity and taxonomic composition of the microbial communities found in each soil sample. Briefly, DNA was extracted using a MoBio PowerSoil extraction kit. For fungi, the first internal transcribed spacer region (ITS1) of the eukaryotic rRNA gene was amplified using the ITS1-F and ITS2 primer pair, and for the prokaryotes, the V4/V5 region of the rRNA gene was amplified using the 515f/806r primer pair. Both the forward and reverse primers also had the appropriate Illumina adapters, primer pad, and 2-bp linker sequences with the reverse primer containing a 12-bp error-correcting barcode unique to each sample. All DNA samples were amplified in triplicate in PCR reactions containing 13 μl water, 10 μl 5 Prime Hot Master Mix, 0.5 μl each of the forward and reverse primers (10 μM final concentration), and 1.0 μl genomic DNA. Reactions were held at 94 °C for 3 min, with amplification proceeding for 35 cycles at 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; a final extension of 10 min at 72 °C. The products of the triplicate PCR reactions were pooled, visualized on an agarose gel, and amplicon concentrations were quantified using the PicoGreen dsDNA assay. Amplicons from all samples were combined in equimolar ratios, and fungi and bacterial/archaeal amplicons were sequenced using two separate runs on an Illumina MiSeq instrument at the University of Colorado at Boulder.

Reads were demultiplexed, quality-filtered, and processed using the QIIME v. 1.6.0-dev pipeline (Caporaso *et al*., 2012). Sequences were clustered into operational taxonomic units (OTUs) using an open reference-based (i.e. reference-based + *de novo*) approach with the UCLUST algorithm (Edgar, 2010) and a 97% similarity threshold. For fungi and prokaryotes, the UNITE and Greengenes (McDonald *et al*., 2012) databases, respectively, were used as reference databases. Samples were rarified to 110 and 4000 sequences for fungi and bacterial/archaea, respectively, prior to downstream analyses. Taxonomy was assigned via the RDP classifier (Wang *et al*., 2007) with the aforementioned databases using a confidence threshold of 0.5.

Catabolic profiling

To compare the relative catabolic abilities of the microbial communities in each of the 66 soils, we used a catabolic profiling assay following (Fierer *et al*., 2011). Briefly, eight different organic carbon substrates (cellulose, chitin, sucrose, glucose, glycine, citric acid, oxalic acid, and yeast extract) were added individually to 4 g (dry wt. equivalent) of each soil. Net CO_2 accumulation was measured on an infrared gas analyzer after an incubation period at 20 °C of 5 or 24 h, depending on the substrate recalcitrance. The substrates were chosen to represent a range of substrates commonly available to soil microorganisms, and included recalcitrant (e.g., chitin, cellulose) and more labile substrates (e.g., amino acids, sugars). Substrates were added at concentrations ranging from 15 to 100 mM in 8 ml of solution with concentrations used in Fierer *et al*. (2011). Assays were conducted in duplicate, yielding 1188 assays in total (66 soils × 8 substrates plus the 'water control' × 2 analytical repeats per substrate per soil). Substrate catabolic rates were calculated as the mean difference from the 'water controls' (those samples that only received water) and were expressed as the mass-specific mineralization (CO_2 production) rates for a given substrate per unit microbial biomass (estimated using PLFA).

Statistical analysis

All multivariate data [microbial community compositions and catabolic response profiles (CRPs)] were analyzed using the PRIMER software package (PRIMER-E, Plymouth, UK). Dissimilarity between microbial community composition and CRPs were calculated using Bray-Curtis and Euclidian distance metrics respectively. Community data were 4th root transformed prior to calculation of community dissimilarities, to minimize the overriding effects of the few most abundant species in each site. Overall effects of site, vegetation type and time on microbial dissimilarity were analyzed using PERMANOVA (based on 999 permutations), and dissimilarities were visualized using principal coordinate analyses (PCoA). Where differences between treatments were significant ($P < 0.05$), taxa responsible for driving changes in community composition were identified using similarity percentage analysis

(SIMPER). Correlations between pairwise dissimilarity among samples (e.g., microbial community composition and CRP dissimilarity) were determined using Mantel tests.

Univariate analyses (biomass, richness, and respiration) were conducted using R version 2.14.2. Multiple Linear Mixed Effects Models were used to explore overall effects of site, vegetation type and time on microbial biomass, diversity, richness, and respiration. They were subsequently used to investigate the dissimilarity in community attributes (composition, biomass, richness) between forest and grassland soil at each site, as a function of *mean* abiotic conditions ('mean annual temperature', 'gravimetric soil moisture', 'pH') organic matter concentrations ('% Carbon', '% Nitrogen', 'C : N ratio'), soil texture ('% sand', '% silt', '% clay'), biome type, and the 'time since vegetation change'. Biotic factors (e.g., forest plant traits or invertebrate communities) were not included because of the practical limitations in capturing a meaning representation of the variation in continent-scale ecosystem types. Our study design therefore included only abiotic factors, allowing prediction of microbial community responses in entirely novel ecosystems, where biotic information is not fully documented or cannot be readily ascertained. For biomass and richness, dissimilarity was calculated by subtracting grassland values from forest values and for community composition, the calculated Bray-Curtis dissimilarity values were used. There was strong autocorrelation in% C and% N between sites so% C was chosen as the best representative variable for organic matter concentration. The variable 'sand : (silt + clay)' was used to represent soil texture because it includes all three particle size-classes, thus incorporates information relating to the total properties of the soil matrix. Conservative (upper bound) *P*-values for the fixed effects were calculated using ANOVA via the LMERConvenienceFunctions package for R.

Results

General effects of forest removal on microbial communities

Despite the substantial temporal variation incorporated into our study design, microbial biomass or richness did not significantly vary within sites with sampling date (Table S2). Instead, vegetation type (forest vs. grassland) had strong and largely consistent directional effects on the biomass and richness of both bacteria and fungi (Table S2). Biomass was generally lower in grassland soil, whereas richness generally increased following forest removal (Fig. 1). The magnitude of the cover effects, however, varied significantly between sites (Site*Cover: $P < 0.001$), with negligible effects in several regions. There were also consistent effects of deforestation on the taxonomic composition (estimated using LUMINA sequencing) of fungal and bacterial communities at the continental scale, even at coarser levels of taxonomic resolution (the phylum-level; Table S2). For bacteria, this community response was driven predominantly by the Acidobacteria (explaining 31.1% of the community difference; SIMPER), and the fungal response was explained by the Basidiomycota (explaining 41.84%), with both of these phyla significantly ($P < 0.05$) less abundant in grassland soils (Fig. 2). To further explore this fungal community response, known fungal genera were identified to specific functional groups [ectomycorrhizal (EM), arbuscular mycorrhizal (AM), and saprotrophic fungi (S)]. Again, there was a significant effect of vegetation type on fungal functional group composition ($F_{1,\ 64} = 28.061$, $P < 0.001$), with a reduction in EM fungi following forest removal explaining 49.04% of the dissimilarity between grassland and forest communities. However, despite these general trends, the effect of vegetation type on bacterial and fungal community composition varied significantly among sites (Cover*Site: $P < 0.001$; Table S2), with dramatic effects in some sites and negligible effects in others (Figure S2).

Legacy effects of forest cover

To explore the site characteristics governing the magnitude of deforestation effects, we regressed microbial community dissimilarity (between forest and grassland) at each site against 'time since vegetation change', mean climate conditions (MAT and MAP), organic matter concentrations (% C and% N), and edaphic characteristics (texture and pH). Soil texture was the only variable to correlate significantly ($P < 0.05$) with the dissimilarity in bacterial biomass, richness, and community composition across sites (Table S3), consistently explaining the vast majority of the variation, with sandier (coarse-textured) soils having a greater dissimilarity following forest removal (Fig. 3). Similarly, soil texture significantly correlated with the susceptibility of fungal biomass, diversity, and community structure to land-use change, with the fungal communities in coarser-textured soils exhibiting greater responses to deforestation than those found in fine-textured soils. To explore the potential generality of texture as a regulator of deforestation responses, we used our models to predict mean values from previous single-site studies, each of which were conducted outside of the USA (Holden & Treseder, 2013; Rodrigues *et al*., 2013). All comparable data published on changes in bacterial biomass, fungal biomass, and bacterial richness fell within the error estimates of our data (Fig. 3).

These mean values represent site characteristics that determine the susceptibility of microbial communities to deforestation. Soil texture did not differ substantially between forest and grassland at any site, but we explored how sand/(silt + clay) correlated with

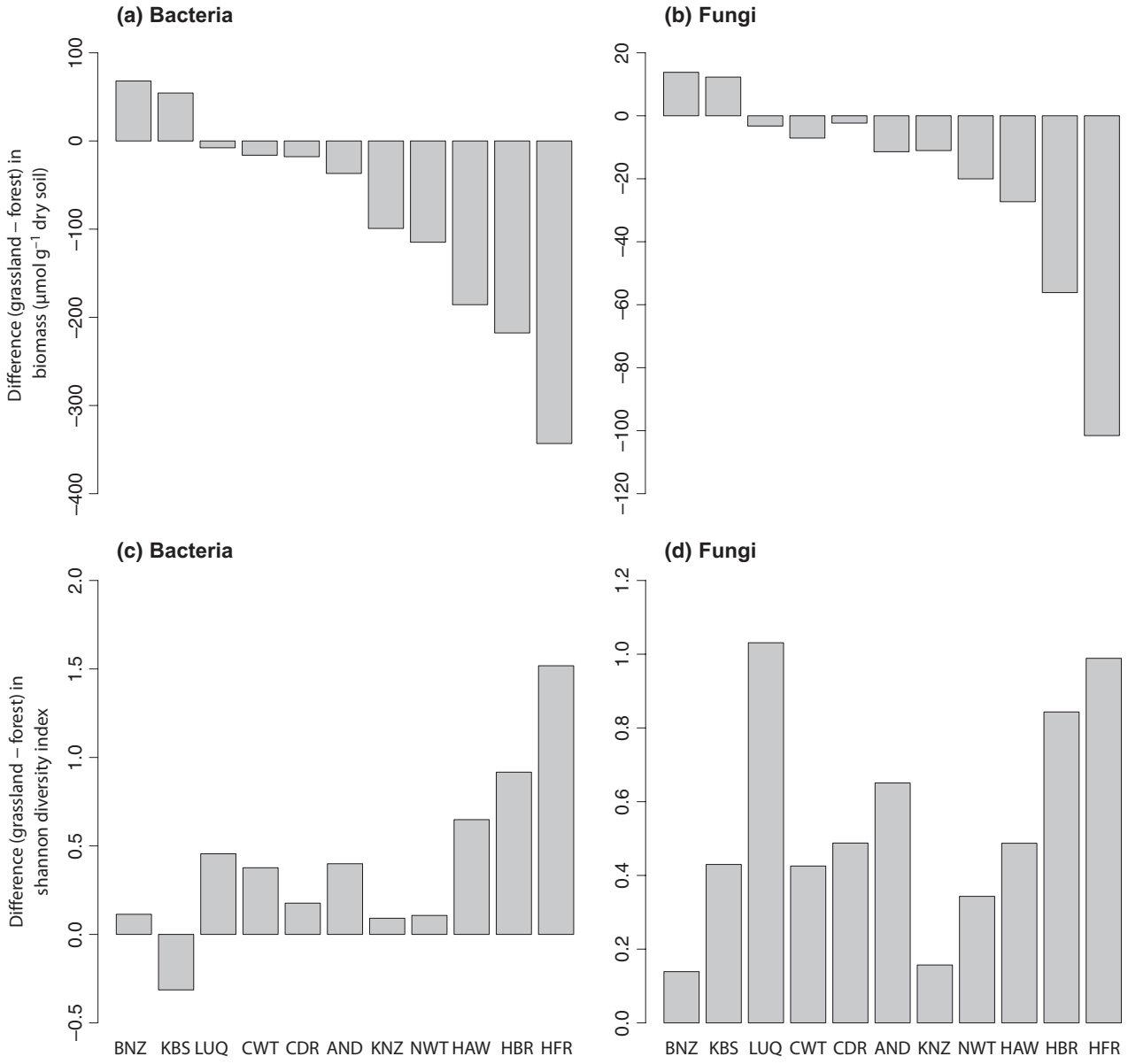

Fig. 1 Differences in the magnitude of effect of forest removal on biomass (estimated using PLFA; a and b) and richness (estimated using Illumina sequencing; c and d) of fungi and bacteria. Microbial OTU richness mirrored Shannon diversity and so is not represented. Sites included 10 long-term Ecological Research stations (Andrews Experimental Forest, Oregon (AND), Bonanza Creek, Alaska (BNZ), Cedar Creek, Minnesota (CDR), Coweeta, North Carolina (CWT), Hubbard Brook, New Hampshire, (HBR), Harvard Forest, Massachusetts (HFR), Kellogg Biological Station, Michigan, (KBS), Konza Prairie Biological Station, Kansas (KNZ), Luquillo, Puerto Rico (LUQ), Niwot Ridge, Colorado (NWT)), and the Hawaii Experimental Tropical Forest, Hawaii (HAW).

'differences' in other environmental characteristics (between forest and grassland) that did vary between forest and grassland sites, to see if it explained where deforestation caused the greatest changes to the soil environment. Difference in pH,% C,% N and gravimetric soil moisture all correlated significantly with sand/(silt + clay) (% C: $r = 0.7$, $P = 0.016$;% N: $r = 0.163$, $P = 0.044$; pH: $r = -0.69$, $P = 0.018$; moisture: $r = 0.8$, $P = 0.003$), with greater effects of vegetation type on all of these soil characteristics with sandier vs. finer-textured soils.

The only metric for which texture was not the strongest explanatory variable was fungal community composition (Table S3). Here, MAT was strongly and negatively correlated with community dissimilarity (Fig. 5). To explore which fungal groups were

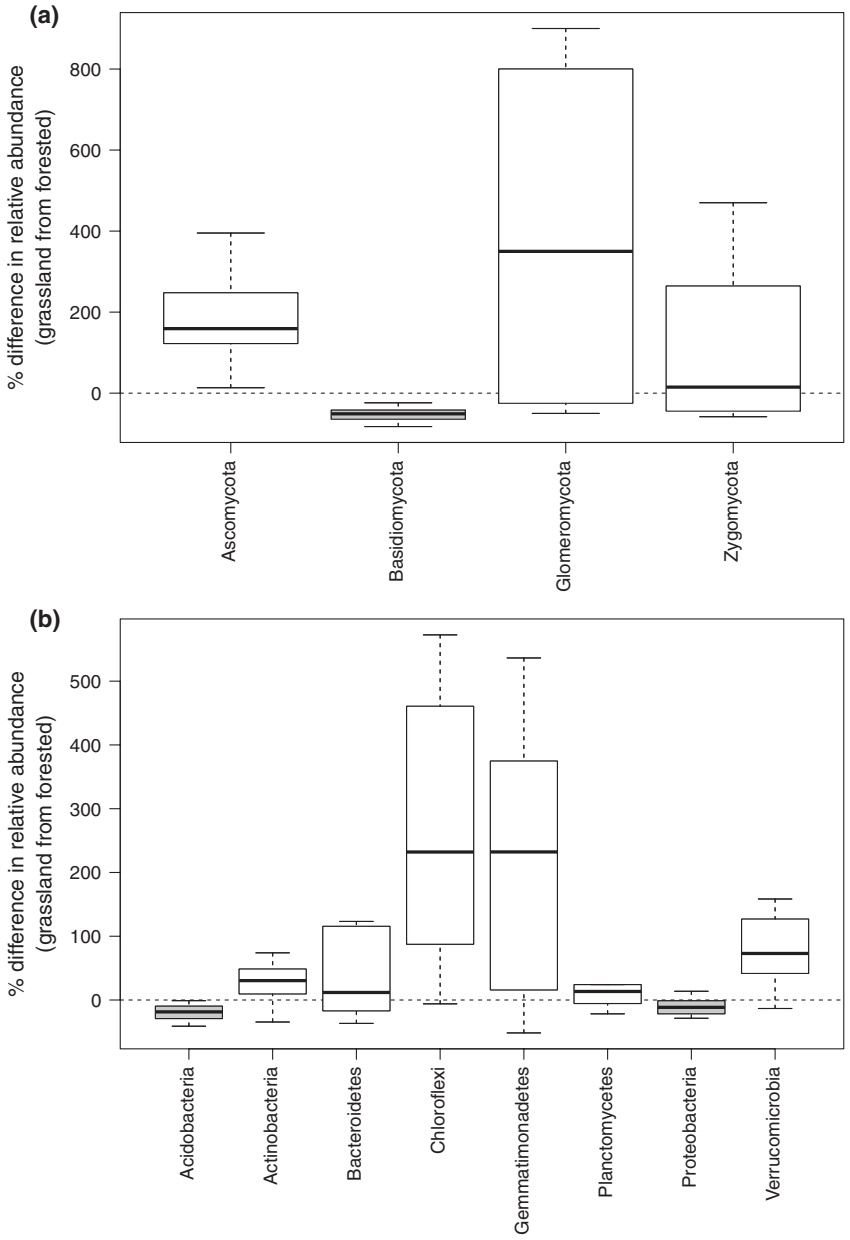

Fig. 2 General changes in microbial community composition (estimated using Illumina sequencing) at the continent-scale. Plots show the mean, interquartile range, and total range of changes (forest – grassland) in relative amlicon abundances of fungal (a) and bacterial (b) phyla following forest removal across 33 soil collections (11 sites over 3 years) across temperate, tropical and boreal sites. Locations are the same as those shown in Fig. 1.

responsible for driving this effect, we regressed the dissimilarity (forest – grassland) in the relative abundance of different fungal groups against MAT. No fungal genera correlated significantly with MAT, but the difference in EM fungi relative abundances decreased significantly ($F_{1,26} = 41.32$, $P < 0.001$) with increasing MAT. The greatest reductions in the relative abundance of EM fungi following forest removal occurred in sites with the coldest temperatures.

Functional responses

In contrast with microbial community structure, the functional attributes of the communities (estimated using catabolic response profiles) did not vary significantly between vegetation types. Instead, substantial temporal variation (sampling date: $P < 0.05$) exceeded the variability between vegetation types (Table S4). The different responses of CRP and community

composition then seem to conflict with recent, site-specific data showing correlations between microbial taxonomic composition and catabolic functioning at finer spatial scales (Fierer *et al.*, 2011). To examine whether this trend holds at the continent-scale, we examined the correlations between phylogeny and functioning among all samples. The catabolic response profiles correlated (MANTEL test; $P < 0.05$) with the taxonomic dissimilarity of both fungi and bacteria. To attribute these functional changes with specific taxonomic changes, we regressed the relative abundances of each fungal and bacterial phylum against mass-specific mineralization rates of individual carbon substrates, following Goldfarb *et al.* (2011). The microbial phyla that consistently changed in response to forest removal were associated with distinct functions; Acidobacteria were associated with significantly greater oxalic acid ($F_{9,54} = 3.476$, $P = 0.004$) and citric acid ($F_{9,54} = 3.476$, $P = 0.017$) mineralization, and increased abundance of Basidiomycetes were correlated with greater mineralization rates of glycine ($F_{9,54} = 2.59$, $P = 0.005$) and cellulose ($F_{9,54} = 2.59$; $P = 0.007$). Overall, although these data show that temporal variation might obscure functional differences between adjacent grassland and forested sites, we provide evidence that catabolic functioning correlates both with community composition and the relative abundance of specific microbial phyla at the broadest spatial scale.

Discussion

We provide a detailed characterization of how both fungal and bacterial communities change following the conversion of forest to grassland, and reveal community-scale trends that hold across tropical, temperate, and boreal biomes. In keeping with previous single-site studies (Da C Jesus *et al.*, 2009; Holden & Treseder, 2013; Rodrigues *et al.*, 2013), forest removal was generally associated with reductions in bacterial biomass and increased diversity. In contrast with theoretical expectations (De Vries *et al.*, 2012a), fungi followed exactly the same trends, with equivalent proportional changes between vegetation types. Despite the substantial diversity of microbial communities within our samples (with well over 1000 microbial OTUs), there was a clear taxonomic signal associated with forest removal at the continent-scale, with consistently lower relative abundances of Acidobacteria (bacteria) and Basidiomycetes (fungi) in grassland soil. These responses are likely driven by the increased recalcitrance of late-succession forest plant material and the acidic nature of forest soils. Specifically, Acidobacteria typically thrive under acidic conditions (Fierer *et al.*, 2012) and basidiomycete fungi are the dominant decomposers of lignocellulose-rich plant material in forests (Hättenschwiler *et al.*, 2005; Crowther *et al.*, 2013). Many of the ectomycorrhizal fungi that colonise the roots of temperate and boreal forest trees also belong to the Basidiomycota, and their absence in grassland soil contributes further the community dissimilarity between vegetation types. These taxonomic differences also exist between vegetation types in the two naturally occurring grassland sites (KNZ and NWT), suggesting that it is likely to be the nature of plant inputs (tree vs. herbaceous) to soils that drive the differences in microbial communities between, rather than the disturbance associated with forest removal. The emergence of this general pattern (that characterize their respective vegetation types) contrasts with previous broad-scale studies, showing no clear differentiation between microbial communities in forest and 'non-forest' soils at the continent-scale (Lauber *et al.*, 2009; Fierer *et al.*, 2012). This highlights the value of pairing forest and grassland sites within biomes to disentangle the effects of vegetation type and abiotic factors at broad spatial scales.

Despite the general differences in belowground communities following deforestation, the magnitude of these differences varied drastically between sites. In contrast with expectations (Holden & Treseder, 2013), 'time since removal' did not affect the magnitude of the community dissimilarity, partially supporting the theory that legacy effects of forest soil can persist for many decades following forest removal in some regions (Gimmi *et al.*, 2012), even if rapid community changes can occur in others. Of the abiotic site characteristics that we measured, only soil texture explained a significant proportion of this variation, with greater community dissimilarity (between vegetation types) in soils characterized by coarse-textured (sandy) soils, and negligible effects in fine-textured (clay) soils (Fig. 3). Belowground responses observed in previous single-site studies were consistent with this trend. For example, in Fazenda Nova Vida (eastern Brazil), mean bacterial richness increased by approximately 47 taxa (OTUs) following forest removal (Rodrigues *et al.*, 2013). This represents a minor response compared to the effects observed at many of our sites, but the magnitude of response was predicted by our data, given that the region is characterized by fine-textured soils (Fig. 3). Although the inclusion of forest plant traits, and other biotic factors are likely to explain some of the residual variation (De Vries *et al.*, 2012b), the strength and consistency of this pattern (for microbial biomass, diversity, and community composition) observed for both bacteria and fungi across multiple biomes suggests the effect of soil texture is highly robust, and can serve as a strong predictor of the responsiveness of microbial communities to land-use change.

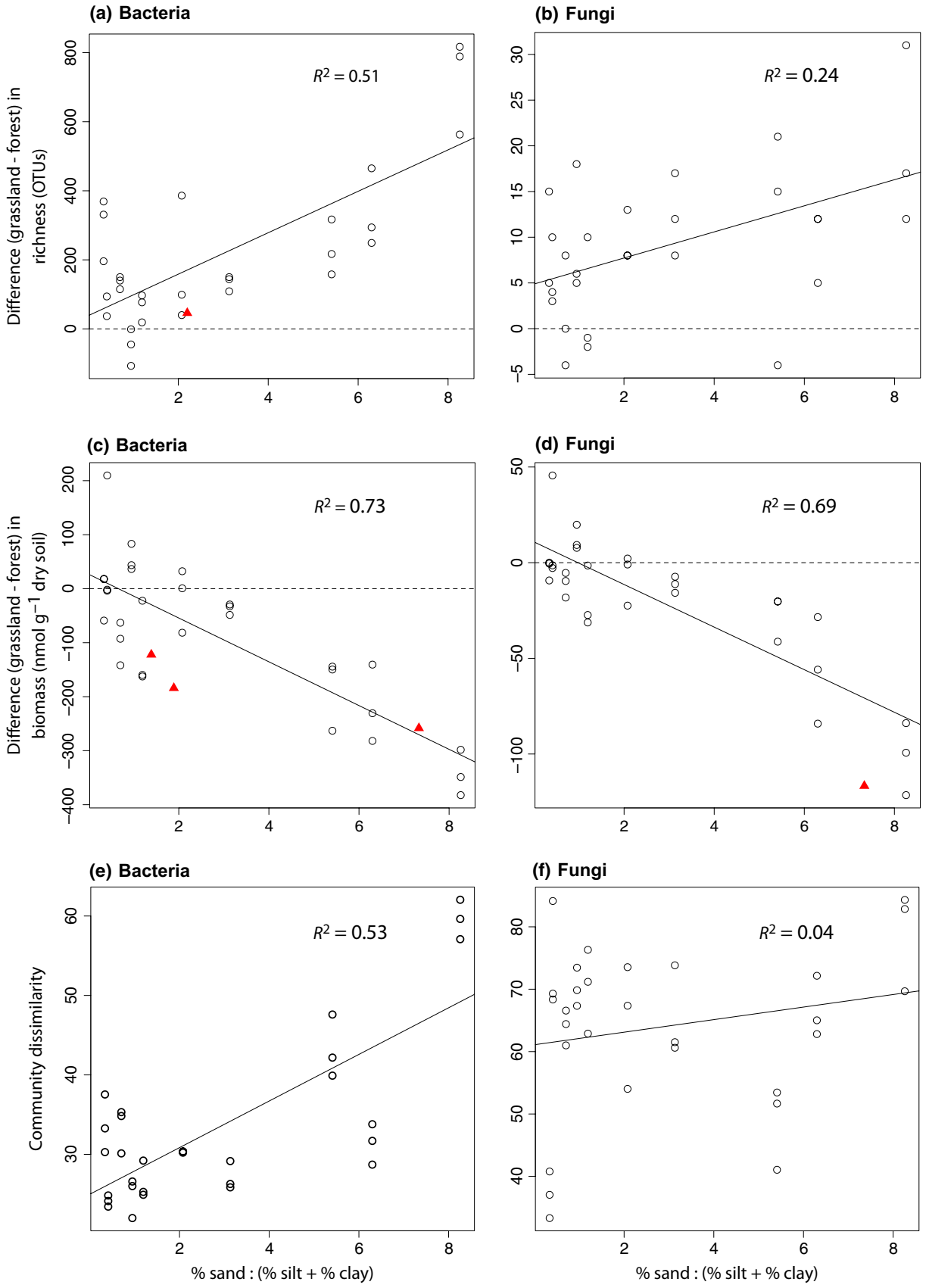
(a) Bacteria
$R^2 = 0.51$
Difference (grassland - forest) in richness (OTUs)
(b) Fungi
$R^2 = 0.24$
(c) Bacteria
$R^2 = 0.73$
Difference (grassland - forest) in biomass (nmol g^{-1} dry soil)
(d) Fungi
$R^2 = 0.69$
(e) Bacteria
$R^2 = 0.53$
Community dissimilarity
% sand : (% silt + % clay)
(f) Fungi
$R^2 = 0.04$
% sand : (% silt + % clay)

Fig. 3 Correlations between dissimilarity (between forest and grassland) in the richness (a and b), biomass (c and d) and community composition (based on calculated Bray-Curtis dissimilarity; e and f) of bacteria and fungi, against soil texture at each site. Regression lines were calculated using linear mixed effects models with 'site' as a random effect and sand/(silt + clay) as the fixed effect. Marginal R^2 values reflect the variance explained by fixed effects only. Models were validated post-hoc, using mean effects recorded in previously published studies (red triangles). Bacterial richness data was collected from Rodrigues *et al*. (2013) and biomass values from Holden & Treseder (2013). The latter were converted from ng g^{-1} soil to nmol g^{-1} soil using the regression equation calculated in Leckie *et al.* (2004). 'Sand : (silt + clay)' was selected to represent soil texture as it incorporates the total properties of the soil matrix. 'Sand : silt' explained more variability, yet it was not used as its biological relevance remains unclear.

The positive effect of silt and clay particles on the resistance of microbial communities to vegetation change presumably results from their high reactive surface area and hence capacity to buffer against changes in soil moisture, pH and nutrient concentrations (Magdoff & Weil, 2004; Grandy *et al.*, 2009). These effects were apparent in our study, as texture correlated with differences in %C, %N, pH, and soil moisture between forest and grassland soils, with greater differences in sandier sites. Loss of organic matter (indicated by the reductions in %C and %N) is thought to be a dominant driver of changes in microbial community attributes following forest removal (Da C Jesus *et al.*, 2009). Our results partially support this conclusion, but suggest that it is ultimately texture that determines the magnitude of these organic matter losses. Fine-textured soils are less well aerated, and nutrients and moisture are protected from decomposition and leaching by being bound in aggregates or through adsorption to the high surface area of clay particles (Magdoff & Weil, 2004; Grandy *et al.*, 2009). The reduced capacity of sand particles (with their small and nonreactive surface areas per volume) to buffer soil characteristics against the effects of forest removal could serve as a guideline for future forest management practices. That is, we might predict that effects of vegetation change on soil biodiversity will be greatest for sandier soils, permitting us to map out microbial vulnerability to vegetation change (e.g., Fig. 4). Notably, the regulatory effect of soil texture was also apparent for sites that included a naturally formed grassland (Fig. 3). We might therefore conclude that texture not only regulates the retention of organic resources in soil, but also the extent to which microbial communities are structured by plant inputs. Recent syntheses argue that plant litter characteristics play a minimal role in structuring microbial community composition in mineral soil (Schimel & Schaeffer, 2012). Our data support this prediction for

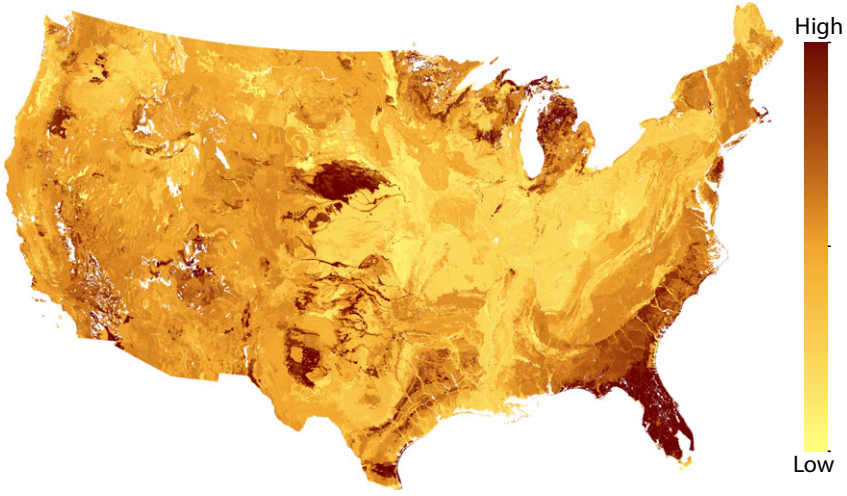

Fig. 4 Heat map representing the susceptibility of total (fungal, bacterial, and archaeal) soil microbial biomass to changes in vegetation cover for the continental United States. White areas represent missing soil texture data. Predicted values were calculated using the fitted mean regression model ($R^2 = 0.70$) given by: Mean change in Biomass = 29.9 − 18.3 × I(Fungi) − 37.2 × sand : (silt + clay) + 27.0 × sand : (silt + clay) × I(Fungi). Where I(Fungi) is a dichotomous indicator variable that equals 1 when the outcome being predicted is 'change in fungal biomass,' and equals 0 when the outcome being predicted is 'change in bacterial biomass.' Soil texture data were obtained from Miller and White (1998). We reiterate that the metric 'sand : silt' explains more variability in microbial biomass than 'sand : (silt + clay)', and, although its biological relevance is unclear, this metric could potentially represent a better metric from a predictive, management perspective.

finer-textured soils where nutrients are physically protected, but suggest that the role of plant inputs is context dependent, with a greater influence on communities in sandier soils.

The only microbial community metric for which soil texture was not the best predictor of vulnerability to deforestation was fungal community structure, which strongly correlated with mean annual temperature. The striking negative effect of temperature on the fungal community differences observed between forest and grassland soils is surprising given that the beta diversity of plants, animals, and bacteria is greater at warmer temperatures (Qian & Ricklefs, 2007; Bryant *et al*., 2008; Korhonen *et al*., 2010). In contrast, we found that fungal community dissimilarity between vegetation types increased with latitude (Fig. 5). This trend may result from the dominance of ectomycorrhizal (EM) fungi (the only mycorrhizal symbionts of boreal forest trees) in north temperate and boreal forests (Clemmensen *et al*., 2013). As obligate symbionts of forest trees, EM fungi are highly susceptible to forest removal and they accounted for 49% of the dissimilarity between vegetation types across our sites. However, their relative abundance decreased at lower latitudes, and there was a negligible effect of vegetation type on the relative abundance of EM fungi in the warmest sites. The genera primarily responsible for the community dissimilarity between forest and grassland soils at the three coldest sites exemplify this trend. The Russula and Cortinarius are predominantly EM fungi, containing species that dominate mature forest soils (Dunham *et al*., 2007; Twieg *et al*., 2007). Both genera were both absent in all tropical and southern temperate soils. The obligate association between EM fungi and host trees at high latitudes may explain the strong effect of temperature on fungal community dissimilarity. Our data then lend support to the growing expectation that biotic interactions are equally, if not more, important than abiotic factors in shaping the structure of soil communities on a continent-scale (Cornwell *et al*., 2009; De Vries *et al*., 2012b).

Although microbial community attributes generally remained constant throughout the 3-year sampling period, their catabolic functioning varied substantially with time. These different temporal dynamics suggest that many species in soil are likely dormant for different parts of the year, while the active proportion varies seasonally (Baldrian *et al*., 2012; Lauber *et al*., 2013). Perhaps surprisingly, given this temporal variation, we found a consistent correlation between microbial taxonomic dissimilarity (fugal and bacterial) and CRP dissimilarity, providing the first evidence for a link between the structure of both bacterial and fungal communities and functional potential at a continent-scale. We stress that is not possible to infer causation from this correlation, but the relationship lends support to a broad suite of local-scale studies that report functional consequences of changes in microbial community composition (Hättenschwiler *et al*., 2005; Fierer *et al*., 2011; Dickie *et al*., 2012; Crowther *et al*., 2013) and suggests that shifts in soil community composition associated

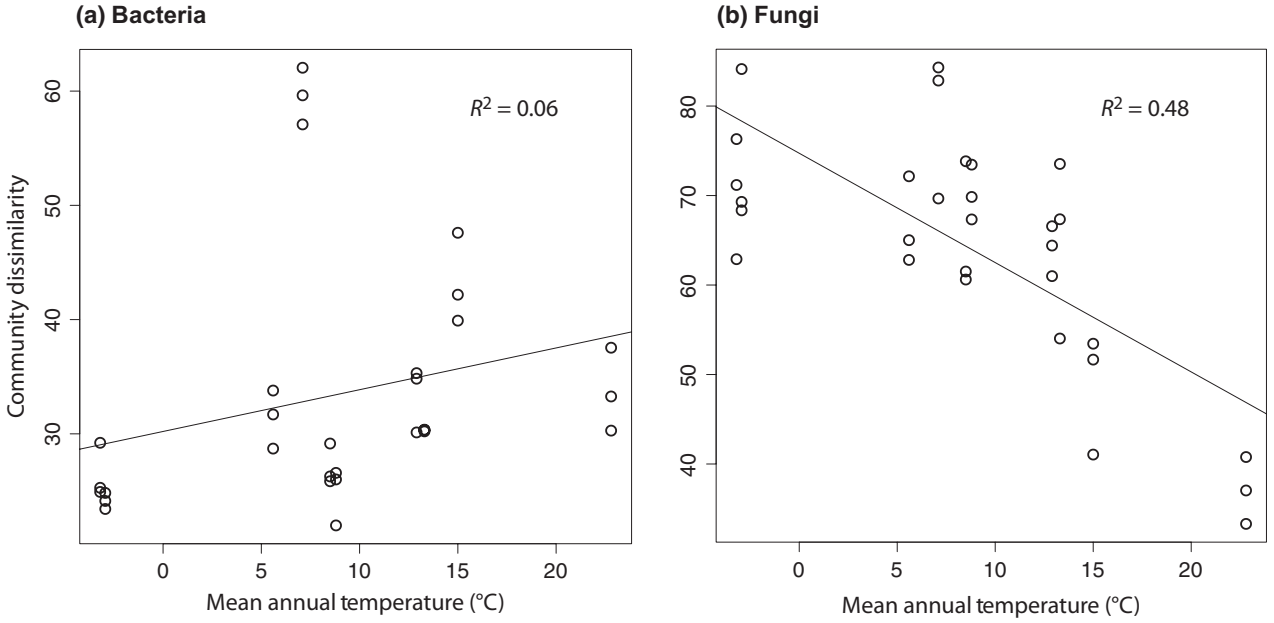

Fig. 5 Effect of mean annual temperature (MAT) on bacterial (a) and fungal (b) community dissimilarity between forest and grassland vegetation types at each site. Regression lines and R^2 values were calculated as in Fig. 3.

with deforestation might be partially responsible for the associated changes in soil C dynamics. Furthermore, correlations between the relative abundance of specific microbial groups and mineralization rates of specific compounds, provides an additional link between microbial response and effect traits. For example, basidiomycetes and Acidobacteria are able to thrive in environments characterized by recalcitrant nutrients and high acidity, and this is reflected in the positive correlation between their relative abundances and the mineralization rates of specific carbon-containing compounds that characterize forest soils. We do not advocate for the scaling up of mineralization rates recorded in such controlled laboratory assays, but these taxon-specific associations with substrate mineralization rates help to provide a mechanistic understanding of the consequences of forest removal for soil organic matter loss.

Conclusions

Understanding the mechanisms that control the extent to which microbial communities change following to vegetation change is essential to comprehend the consequences of deforestation for belowground biodiversity and carbon cycling (Sala, 2000; Van der Werf *et al.*, 2009). Despite the complex nature of soil microbial communities, we found general patterns characterizing microbial community responses to deforestation at the continental scale. The magnitude of these responses were, however, highly variable and strongly influenced by soil texture, which regulates the extent of soil organic matter losses following deforestation. Specifically, forest removal dramatically alters the structure of belowground communities in sandier soils, whereas finer-textured soils buffer microbial communities against the effects of land-use change. Other site characteristics were less important in structuring microbial responses, except with respect to fungal community composition, where increasing mean annual temperature decreased the magnitude of the response to forest removal. The belowground community responses were associated with distinct changes in microbial catabolic profiles, highlighting direct functional consequences of the belowground community change. The predictable nature of the microbial community responses to vegetation type opens the door for a new generation of terrestrial models that help anticipate the consequences of land-use change for the biodiversity and carbon storage of forest ecosystems worldwide.

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Authorship Statement

TWC, MAB, NF, and RLM conceived specific question and study design. TWC, MAB, and EEO performed soil analyses. JWL and NF conducted high-throughput sequencing and data collection. RLM conducted PLFA analysis and interpretation. DSM, TWC, and JWL conducted statistical analysis and TWC wrote the study with contribution from all authors.

References

Aldrich-Wolfe L (2007) Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. *Ecology*, **88**, 559–566.

Baldrian P, Kolařík M, Stursová M *et al.* (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal*, **6**, 248–258.

Bouyoucos GJ (1927) Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal*, **54**, 464–465.

Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL (2008) Colloquium paper: microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11505–11511.

Caporaso JG, Lauber CL, Walters WA *et al.* (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal*, **6**, 1621–1624.

Clemmensen KE, Bahr A, Ovaskainen O *et al.* (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, **339**, 1615–1618.

Cornwell WK, Cornelissen JHC, Allison SD *et al.* (2009) Plant traits and wood fates across the globe: rotted, burned, or consumed? *Global Change Biology*, **15**, 2431–2449.

Crowther TW, Bradford MA (2013) Thermal acclimation in widespread heterotrophic soil microbes. *Ecology Letters*, **16**, 469–477.

Crowther TW, Stanton DW, Thomas SM *et al.* (2013) Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology*, **94**, 2518–2528.

Da C Jesus E, Marsh TL, Tiedje JM, de S Moreira FM (2009) Changes in land use alter the structure of bacterial communities in Western Amazon soils. *The ISME Journal*, **3**, 1004–1011.

De Vries FT, Liiri ME, Bjørnlund L, Bowker MA, Christensen S, Setälä HM, Bardgett RD (2012a) Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change*, **2**, 276–280.

De Vries FT, Manning P, Tallowin JRB *et al.* (2012b) Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters*, **15**, 1230–1239.

Dickie IA, Fukami T, Wilkie JP, Allen RB, Buchanan PK (2012) Do assembly history effects attenuate from species to ecosystem properties? A field test with wood-inhabiting fungi. *Ecology Letters*, **15**, 133–141.

Dinesh R, Ghoshal Chaudhuri S, Ganeshamurthy AN, Dey C (2003) Changes in soil microbial indices and their relationships following deforestation and cultivation in wet tropical forests. *Applied Soil Ecology*, **24**, 17–26.

Dunham SM, Larsson K-H, Spatafora JW (2007) Species richness and community composition of mat-forming ectomycorrhizal fungi in old- and second-growth Douglas-fir forests of the HJ Andrews Experimental Forest, Oregon, USA. *Mycorrhiza*, **17**, 633–645.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, **26**, 2460–2461.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2011) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal*, **6**, 1007–1017.

Fierer N, Leff JW, Adams BJ *et al.* (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences of the USA*, **109**, 21390–21395.

Findlay RH, Dobbs FC (1993) Quantitative description of microbial communities using lipid analysis. In: *Current methods in aquatic microbial ecology* (ed. Kemp PF), pp. 271–284. Lewis Publications, Boca Raton, FL.

Gimmi U, Poulter B, Wolf A, Portner H, Weber P, Bürgi M (2012) Soil carbon pools in Swiss forests show legacy effects from historic forest litter raking. *Landscape Ecology*, **28**, 835–846.

Goldfarb KC, Karaoz U, Hanson CA, *et al.* (2011) Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Frontiers in Microbiology*, **2**, 94.

Grandy AS, Strickland MS, Lauber CL, Bradford MA, Fierer N (2009) The influence of microbial communities, management, and soil texture on soil organic matter chemistry. *Geoderma*, **150**, 278–286.

Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 191–218.

Holden SR, Treseder KK (2013) A meta-analysis of soil microbial biomass responses to forest disturbances. *Frontiers in microbiology*, **4**, 1–17.

Johnson DW, Curtis PS (2001) Effects of forest management on soil C and N storage: meta analysis. *Forest Ecology and Management*, **140**, 227–238.

Korhonen JJ, Soininen J, Hillebrand H (2010) A quantitative analysis of temporal turnover in aquatic species assemblages across ecosystems. *Ecology*, **91**, 508–517.

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, **75**, 5111–5120.

Lauber CL, Ramirez KS, Aanderud Z, Lennon J, Fierer N (2013) Temporal variability in soil microbial communities across land-use types. *The ISME Journal*, **7**, 1641–1650.

Leckie SE, Prescott CE, Grayston SJ, Neufeld JD, Mohn WW (2004) Comparison of chloroform fumigation-extraction, phospholipid fatty acid, and DNA methods to determine microbial biomass in forest humus. *Soil Biology and Biochemistry*, **36**, 529–532.

Magdoff F, Weil RR (2004) *Soil organic matter in sustainable agriculture.* CRC Press, Boca Raton, FL.

McDonald D, Price MN, Goodrich J *et al.* (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal*, **6**, 610–618.

Miller DA, White RA (1998) A conterminous United States multilayer soil characteristics dataset for regional climate and hydrology modeling. *Earth interactions*, **2**, 1–26.

Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011) How many species are there on Earth and in the ocean? *PLoS Biology*, **9**, e1001127.

Öpik M, Zobel M, Cantero JJ *et al.* (2013) Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza*, **23**, 411–430.

Pan Y, Birdsey R a, Fang J *et al.* (2011) A large and persistent carbon sink in the world's forests. *Science*, **333**, 988–993.

Qian H, Ricklefs RE (2007) A latitudinal gradient in large-scale beta diversity for vascular plants in North America. *Ecology Letters*, **10**, 737–744.

Rodrigues JLM, Pellizari VH, Mueller R *et al.* (2013) Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 988–993.

Sala OE (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.

Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, **3**, 348.

Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytologist*, **176**, 437–447.

Van der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.

Van der Werf GR, Morton DC, DeFries RS *et al.* (2009) CO 2 emissions from forest loss. *Nature Geoscience*, **2**, 9–11.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, **73**, 5261–5267.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean site characteristics.
Table S2. Effect of sample time and location on microbial community attributes.
Table S3. Factors affecting microbial susceptibility to deforestation.
Table S4. Effect of sample time and location on the carbon mineralization indices**.**
Figure S1. Map displaying the locations and the forest characteristics of each site.
Figure S2. Bacterial (a) and fungal (b) community composition in 66 soil samples collected from 22 sites across 3 years.