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Short Communication

Post-agricultural tropical forest regeneration shifts soil microbial functional potential for carbon and nutrient cycling

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ABSTRACT

Natural forest regeneration on abandoned agricultural land is an increasingly common, but understudied, landcover change in the tropics. We studied whether observed changes in microbial community composition with forest regeneration led to changes in functional potential. We used GeoChip 3.0 to measure microbial functional potential for carbon (C), nitrogen (N), phosphorous (P), and sulfur (S) cycling genes in pastures, early successional, and late successional forests in Puerto Rico. We found lower abundance and diversity of genes involved in C, N, and P in late successional forests compared to other landcover types. Soil microbial communities were functionally similar in active pastures and early successional forests. Our results suggest microbial functional potential varies with forest age, with potential implications for tropical biogeochemistry.

Soil microorganisms play key roles in regulating ecosystem productivity through decomposition, nutrient cycling, and soil organic matter formation (Van Der Heijden et al., 2008; Cotrufo et al., 2013; Kallenbach et al., 2016). Despite the importance of microorganisms in biogeochemistry, their recovery from disturbance remains poorly understood (Kuramae et al., 2010; Banning et al., 2011), especially in tropical forests, where post-agricultural natural forest regeneration is a dominant, but understudied, landcover change (Powers and Marín-Spiotta, 2017). Previous work in tropical forests in Puerto Rico found successional shifts in plant and soil microbial communities with post-agricultural forest regeneration (Marin-Spiotta et al., 2007; Smith et al., 2015). We studied whether observed changes in microbial community composition led to changes in functional potential. We investigated differences in abundance and diversity of microbial C, N, phosphorous (P), and sulfur (S) cycling genes between post-agricultural successional tropical forest landcover types in southeastern Puerto Rico.

We expected higher gene abundance and diversity in regenerated forests than in pastures due to more plant species and divergent plant litter chemistries (Marín-Spiotta et al., 2008). Plant litter inputs were dominated by simple carbohydrates in pastures and chemically complex substrates in successional forests (Marín-Spiotta et al., 2008). Therefore, we hypothesized that the capacity of microbial communities for lignin degradation will increase, whereas cellulose degradation will decrease with forest regeneration. We also hypothesized that microbial N fixation potential follows a hump-shaped pattern with the highest abundance and diversity of N fixation genes in early successional forests because the abundance of N-fixing trees and N fixation rates are typically highest in early successional tropical forests (Gehring et al., 2005; Powers and Marín-Spiotta, 2017). Total soil P does not vary with secondary forest regeneration (Powers and Marín-Spiotta, 2017) and little is known about S dynamics in post-agricultural tropical forests. Therefore, we hypothesized that microbial communities would be functionally similar with respect to P and S cycling between forest regeneration landcover types.

In January 2012, we collected soils to a depth of 20 cm from 4 replicates of pastures, early (40-year), and late (90-year) successional

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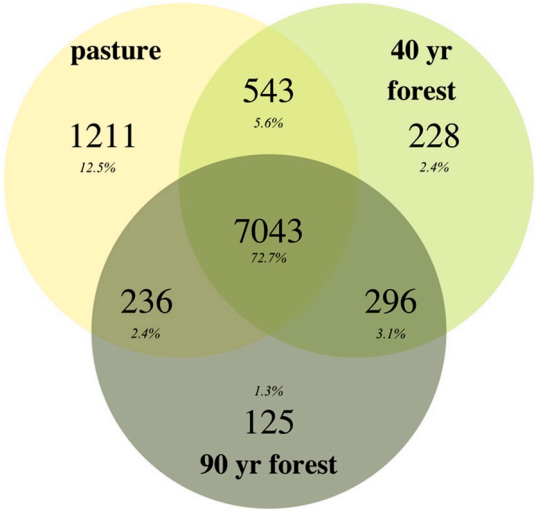

Fig. 1. Venn diagram of GeoChip 3.0 probe overlap of functional genes involved in C, N, P, and S cycling between post-agricultural successional tropical forest regeneration landcover types. Numeric value indicates number of shared probes; percentage of total probes shown in italics. Individual Venn diagrams for C, N, P, and S cycling probes can be found in Supplementary 3.

subtropical forests in Sierra de Cayey, Puerto Rico. Detailed description of field sites is in Marín-Spiotta et al. (2007, 2008), and Smith et al. (2014, 2015). We quantified microbial functional potential using GeoChip 3.0, a functional gene microarray that uses signal intensity to estimate gene abundance (He et al., 2007, 2010; Zhou et al., 2010). In R (version 3.4.3), we calculated total signal intensities to represent functional potential within each functional category, process, or gene. We calculated Shannon's diversity (H) on the number of probes present for each functional process (e.g. acetogenesis) using the diversity function from the vegan package (Oksanen et al., 2019). We ran fixed effects models and ANOVA with Tukey adjustment with total signal intensity and Shannon's diversity as response variables and landcover and site as predictors. We corrected for multiple comparisons using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995). Effects were only marginally significant after correction ($p < 0.15$) and we discuss these trends because they serve as important starting points for future research. Additional details on sampling, GeoChip analysis, data summarization, and statistics are in Supplemental 1.

Soil microbial functional potential for C, N, and P cycling was lowest in late successional forests, but similar in pastures and early successional forests. We observed the greatest overlap in microbial functional potential between pastures and early successional forests (Fig. 1; Supplementary 3). We found marginal differences in beta-diversity of total signal intensities for C, N, and S cycling probes among landcover types using nonparametric multidimensional scaling and nonparametric multivariate dissimilarity tests (Supplementary 2). Total signal intensities (Fig. 2a; Table 1) and diversity (Fig. 3) for cellulose, hemicellulose, and chitin degradation-related probes were marginally lower in late successional forests compared to other landcover types. Our results do not support the hypothesis that forest microbial communities will have a higher capacity for lignin degradation because the abundance and diversity of lignin degrading genes (glyoxal oxidase, lignin peroxidase, manganese peroxidase, and phenol oxidase) was not significantly different between landcover types (Figs. 2a and 3). Microbial communities may have a reduced capacity for cellulose and hemicellulose degradation in late successional forests due to fewer labile, carbohydrate-rich C inputs from tree roots relative to grasses and forbs characteristic of pastures. However, total soil C and C:N did not differ with landcover type (Smith et al., 2014) and leaf and root litter decomposition showed no predictable pattern with forest age (Ostertag et al., 2008). The abundance and diversity of lignin-degrading genes remained similar between landcover types, despite lower PLFA-determined fungi:bacteria ratios in late successional forests (Smith et al., 2014, 2015). This pattern could be due to functional redundancy in the fungal community and the presence of lignin-degrading bacteria in these tropical forest soils (DeAngelis et al., 2011).

Though total soil N did not differ between landcover types (Smith et al., 2014), total signal intensities for N fixation, ammonification, dissimilatory nitrate reduction to ammonium (DNRA), and bacterial nitrification gene probes were lower in late successional forests compared to other landcover types (Fig. 2b, Table 1). Functional diversity of DNRA, bacterial nitrification, and N fixation genes was also lowest in late successional forests (Fig. 3). Although we did not observe the expected hump-shaped response, our results provide some support for the hypothesis of lower N-fixation potential in late successional forests. The decrease in N-fixation potential is likely driven by a lower abundance of N-fixing trees in mature tropical forests (Gehring et al., 2005; Powers and Marín-Spiotta, 2017).

Total signal intensities (Fig. 2b) and relative diversity (Fig. 3) for P-degrading genes were lowest in late successional forests compared to other landcover types (Fig. 2b). This was driven by a decrease in *ppx*, which encodes exopolyphosphatase for inorganic polyphosphate degradation (Table 1). Soil microbial communities may have a reduced capacity for P cycling with forest regeneration because older successional tropical forests have conservative P biogeochemistry and are more P limited than early successional forests (Davidson et al., 2007). Therefore, microbial communities may invest less in P-degrading genes as P becomes limiting with forest age and decreasing substrate availability. However, the opposite has also been observed. Soil microbial communities in a subtropical peatland invested more in P acquisition relative to N acquisition as P became limiting (Morrison et al., 2016). Our results provide some support to the former mechanism.

Soil microbial functional potential for S cycling was similar across landcover types. Total signal intensities (Fig. 2b) and relative diversity (Fig. 3) for S cycling genes were not significantly different between landcover types. Likewise, extracellular enzyme activities were not significantly different between landcover types (Smith et al., 2015). Functional similarities in microbial sulfur cycling potential could be due to comparable soil S concentrations between landcover types, as was found in analogous tropical forests in Panama (Yavitt, 2000). However, soil S can vary greatly with elevation Puerto Rican tropical forests (Stanko-Golden and Fitzgerald, 1991).

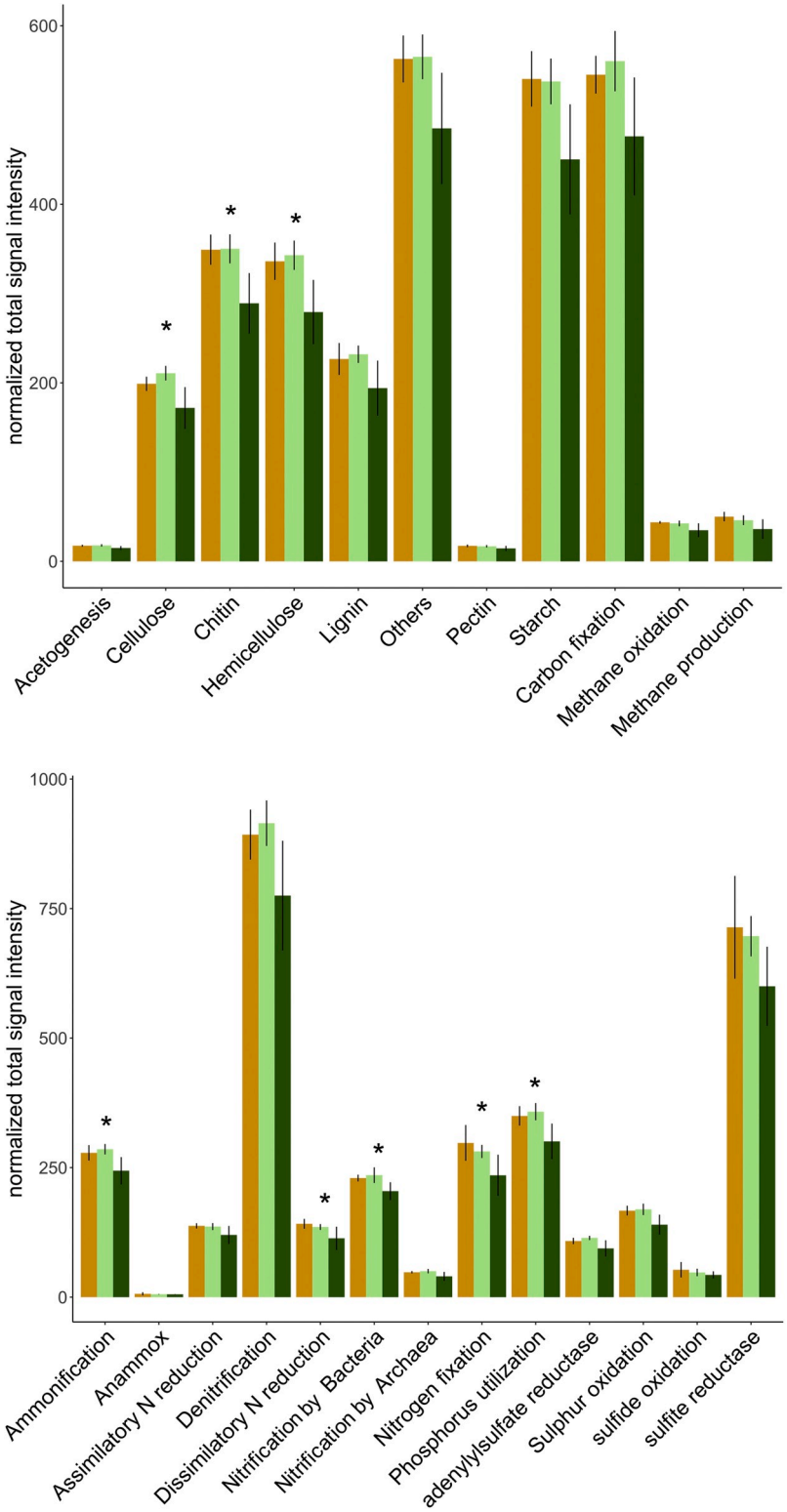

Fig. 2. Total signal intensities of detected probes involved in carbon cycling processes (a), and nitrogen, phosphorus and sulfur cycling processes (b) between forest regeneration landcover types (n = 4). Asterisks (*) indicate late successional forests are significantly different ($p < 0.05$) prior to FDR correction and marginally significant ($p < 0.15$) after correction. Total signal intensities were derived by summing the signal intensity of detected probes within a functional process (e.g. acetogenesis). A full description of data normalization for the signal intensities can be found in Supplementary 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Genes and enzymes with significantly lower total signal intensities in late successional forests ($p < 0.05$) prior to FDR correction and marginally significant ($p < 0.15$) after correction, compared to early successional forests and pastures ($n = 4$).

Process	Gene/enzyme
Cellulose degradation	*egl*, endoglucanase
	exoglucanase
Chitin degradation	endochitinase
	acetylglucosaminidase
Hemicellulose degradation	*ara*, arabinofuraosidase
Starch degradation	*pulA*, pullulanase
	nplT, neopullulanase II
	cda, cyclomaltodextrin dextrin-hydrolase
Other C degradation	*vanA*, vanillate monoogygenase
Ammonification	*gdh*, glutamate dehydrogenase
N fixation	*nifH*, dinitrogenase reductase
P utilization	*ppx*, exopolyphosphatase
Sulfite reduction	*dsrA*, dissimilatory sulfite reductase subunit A

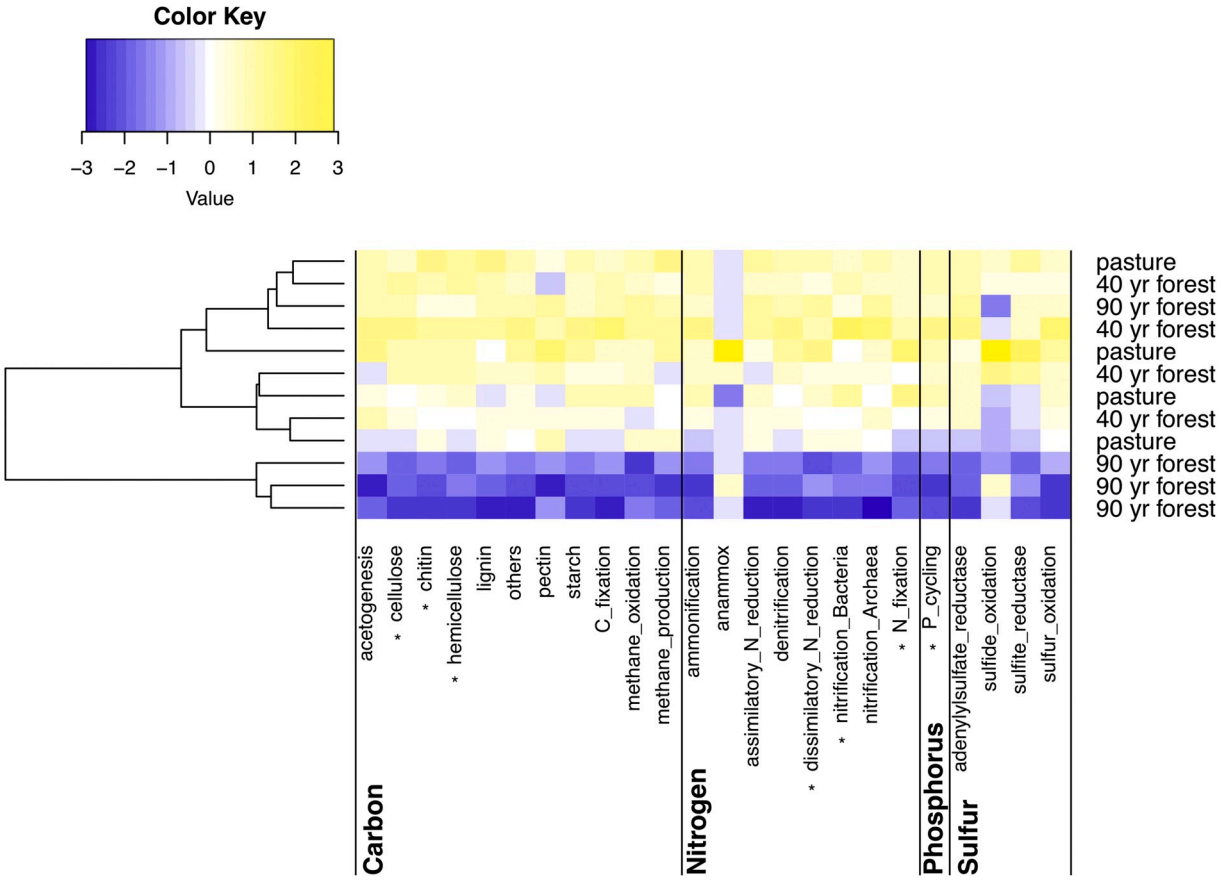

Fig. 3. Cluster analysis and heat map of relative functional diversity (Shannon's H′) of probes for genes involved in C, N, P, and S cycling processes from soil microbial communities from different forest regeneration landcover types ($n = 4$). Darker colors indicate lower relative diversity of genes within each functional process whereas lighter colors indicate higher relative diversity. Asterisks (*) indicate significantly lower diversity in late successional forests (90 yr forest; $p < 0.05$) prior to FDR correction and marginally significant ($p < 0.15$) after correction. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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.2020.107784.

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