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Effects of natural and experimental drought on soil fungi and biogeochemistry in an Amazon rain forest

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Microbiota are essential components of the soil, driving biogeochemical cycles. Fungi affect decomposition and biotic interactions with plants across scales. Climate projections suggest that extended dry seasons may transform sensitive rain forests into savanna-like vegetation, with consequent changes in biogeochemistry. Here we compare the impacts of natural seasonality with 14 years of partial throughfall exclusion in an Amazonian rain forest, focussing on soil fungal functional diversity, extracellular soil enzyme activities (EEA) and their implications for nutrient dynamics. Large changes in fungal diversity and functional group composition occur in response to drought, with a conspicuous increase in the abundance of dark-septate fungi and a decrease in fungal pathogens. The high seasonality of EEA in the control (non droughted) and suppression of seasonality in the drought treatment, together with an increased implied nitrogen demand in the dry season induced by experimental drought, suggest that the changed soil microbiota activity may signal a pending shift in the biogeochemical functioning of the forest.

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Forest conversion in Amazonia has reached *ca*. 20% and it is estimated that an additional 20–25% of forest loss could permanently transform the regional climate of the basin and the remaining forest vegetation[1]. In addition, drought frequency, duration and severity are predicted to increase pan-tropically, particularly in Amazonia, and impact rain forests as a result of global climate change this century[2–4]. These regional and global changes in climate could, in the long term, transform large portions of the remaining evergreen Amazonian rain forests into a seasonal savanna-type vegetation or into seasonal and dry forests[5–7], modifying biogeochemical cycles substantially[8,9] and compromising the provisioning of ecosystem services[10].

Numerous recent works have investigated via observation[11,12], modelling[5,13,14] and experimentation[15], the impacts of drought on tropical rain forests[16,17] and their carbon (C) sequestration potential[18]. The principal approaches have examined ecophysiological functioning[19,20] and demographic parameters (stem death and recruitment rates)[21,22], important for both short-term acclimation, and for longer-term community change and adaptive evolution. While additional studies have also focused on litterfall[23] and soil respiration[17,24], the effects of long-term drought on microbe-driven biogeochemical cycles have not yet been investigated. How such impacts on nutrient cycling may be offset by novel soil microbial communities that are able to elicit functional changes is a little explored subject, especially in tropical rain forests. This is a major lacuna in our understanding, as drought has direct consequences for, and feedback from, likely changes in microbial community composition, for biotic interactions between trees and microbes, and among microbes.

Fungal communities are fundamental for soil and ecosystem functioning through their contribution to processes, such as organic matter decomposition, nutrient cycling and storage[25]. They can be directly affected by drought, or indirectly by shifts in plant community structure and/or physiology in response to the modified conditions[26]. Although soil fungal communities have been shown to shift seasonally in tropical rain forests[27], the functional groups that drive the fungal response to long-term drought and the consequences on biogeochemical cycles remain unknown. Soil microbial communities produce extracellular enzymes involved in the turnover of C and nutrients from organic compounds, and ratios of potential soil extracellular enzyme activity (EEA) are used to infer microbial C, nitrogen (N) and phosphorus (P) demand[28], and provide important information on biogeochemical cycles, for which information is relatively limited in tropical soils[29,30].

Microbial enzyme allocation in tropical ecosystems is coupled to soil nutrient stoichiometry and availability, and is tightly connected with temperature and precipitation[31]. At the local scale, for example, EEA is strongly affected by seasonal precipitation variability with lowest activities in the dry season[29,32,33]. Soil moisture is an important variable in biogeochemical processes mediated by microbial communities, and represents a reliable proximal control on the rates of microbial function, such as respiration[34,35] or nitrification[36]. The mechanisms that underlie links between soil moisture and EEA may include direct effects of water availability on microbial activity or indirect effects related to changes in oxygen levels[37,38] and in the flux of nutrients and labile C[39,40]. To improve our ability to estimate how projected climate change (frequency of drought events—pulse-type disturbances vs. prolongation of the dry season—press-type perturbations) may alter microbially-mediated biogeochemical cycles, we need to understand how potential large changes in soil moisture could affect EEA, shape soil fungal communities, and alter their functional diversity (here defined by functional groups), which together are in direct interplay with biogeochemical processes[41,42]. For example, major shifts in soil fungal functional or trophic groups may occur, which can reconfigure plant–microbe interactions and nutrient cycling and water acquisition.

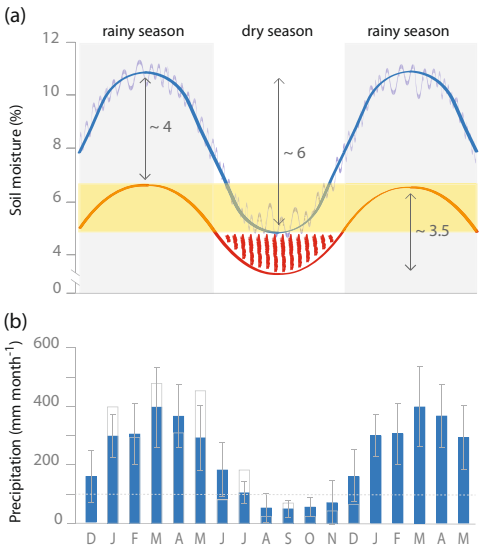

Fig. 1 Conceptual representation of seasonal and experimental variation in soil moisture in a lowland Amazonian rain forest on sandy soil. a The amplitude of natural seasonally occurring variation in soil moisture content (control, blue) contrasted with that of the drought treatment (~50%) after 14 years of throughfall exclusion in a lowland evergreen rain forest in Amazonia. Soil moisture in the drought treatment during the rainy season (orange) is within the range of naturally occurring variation (referred to as non-extreme drought); however, it falls below this range in the dry season (extreme drought, red). The natural inter-annual variability in soil moisture is represented by the sinusoidal curve in grey (non-extreme drought). Values indicate the average percentage of soil moisture in control and treatment at the time of soil sampling. **b** Average annual monthly rainfall and standard deviation measured at the study site during the period 2001–2016 (filled) and for the year 2016 (unfilled). Grey dotted line represents the monthly precipitation required for hydrological balance, considered 100 mm in Amazonia.

Long-term observations and experiments are useful for documenting environmental fluctuations and extremes, and ecosystem responses to them[43]. The availability of long-term data allow the interpretation of short-term observations in a temporal context, such as if the response is attributable to treatment, or likely to be affected by cyclic fluctuations or recovery from extreme events[43]. Long-term studies may be combined with the imposition of large environmental manipulations on the system[44] to study ecosystem responses and test hypotheses that modelling alone is unable to address. One such rare experiment is the long-term canopy throughfall exclusion experiment in the Caxiuanã National Forest Reserve, in eastern Brazilian Amazonia, established in 2001 to study the response by an Amazon rain forest to substantial soil drought, but within the bounds of climate predictions for this century[15]. The exclusion of about 50% of the throughfall throughout the year (Fig. 1) which at the time of this study had reduced available soil water for trees and microbes over a period of 14 years, represents an ecosystem that after major structural and functional changes (in response to the initial pulse disturbance and subsequent extended drought), today allows the separation of the short-term fluctuations in soil moisture availability caused by precipitation seasonality, from long-term press-type perturbation caused by long-term throughfall exclusion[15]. At this point in time it is valid to ask what changes, if any, in the biotic community, might have contributed to modulating the

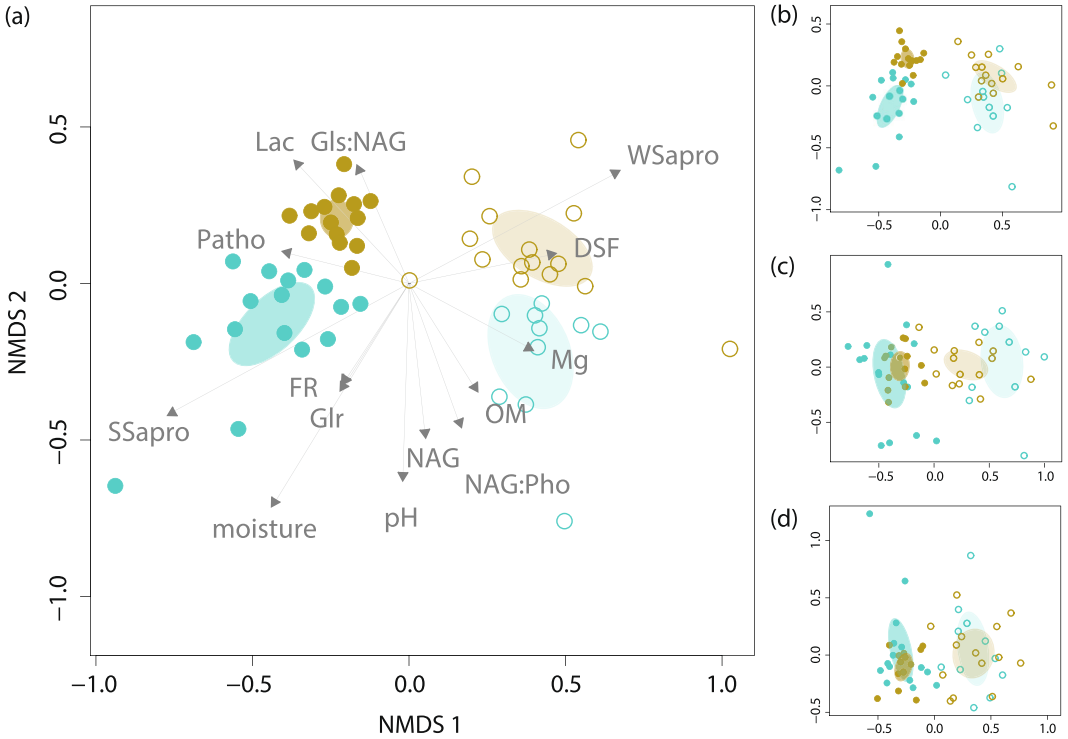

Fig. 2 Change in Amazon rain forest soil fungal communities in response to precipitation seasonality and experimental drought. Plots of non-metric multidimensional scaling (NMDS) ordination based on non-rarefied presence/absence data of fungal communities in response to seasonality and 14 years of throughfall exclusion (~50%) in a tropical lowland rain forest in the Amazon basin. **a** Total fungi; stress 2D, 0.127; **b** saprotrophs; stress 2D, 0.163; **c** dark-septate fungi; stress 3D; 0.166; **d** pathogens; stress 3D, 0.162. Green, wet season; brown, dry season; filled circles, control; empty circles, treatment; DSF dark-septate fungi, Patho pathogens, SSapro soil saprotrophs, Wsapro wood saprotrophs, OM soil organic matter, FR fine roots, Mg magnesium, Glr ß-Glucuronidase, Gls ß-Glucosidase, Lac Laccase, NAG N-Acetylglucosaminidase, Pho Acid phosphatase. Ellipses denote 95% confidence intervals using standard error of the weighted average sample scores per treatment in the rainy and dry seasons.

functioning of the forest in the drought treatment, through compensatory mechanisms for nutrient and water acquisition, likely mediated by biotic interactions not accounted for in previous studies that have not considered the microbiology of the soil.

We hypothesized that (H_{1A}) the natural precipitation seasonality would increase fungal taxonomic diversity during the dry season in the control relative to the rainy season (as per the intermediate disturbance hypothesis, IDH), as found elsewhere in tropical evergreen rain forests[27,45,46]. We also hypothesised that the IDH would hold for the throughfall exclusion treatment vs. control in the rainy season with an increased taxonomic diversity in the treatment (it being within the annual range of soil moisture fluctuation, at similar level to that in the dry season in the control). Conversely (H_{1B}), the long-term severe soil drought, produced in the throughfall exclusion treatment, and acting as a major press-type disturbance, would be expected to reduce fungal taxonomic diversity in the dry season and select for specific functional groups that are naturally abundant during the normal dry season. Finally, we expected (H_2) a reduction in EEA under seasonal drought conditions[32] in (i) the control; (ii) in the throughfall exclusion treatment in the rainy season relative to the control; and (iii) a larger reduction in the drought treatment during seasonal drought relative to the control. To test our hypotheses 25 soil samples were collected at the peaks of the rainy and dry seasons in each of the 1-ha rainfall exclusion treatment and in its paired control plot. Soil samples were analysed for diversity and composition of soil fungi and functional groups and EEA. The data were used to examine the effects of non-extreme drought that falls within the natural range of observed variability (in the short term, between wet and dry seasons in the control; and in the throughfall exclusion treatment in the wet season) and a condition which falls outside of the natural throughfall variability (extreme drought) experienced by this tropical rain forest and is only produced in the long-term throughfall exclusion treatment during the dry season (Fig. 1).

Major changes in fungal diversity, functional group composition and EEA are observed in response to natural precipitation seasonality and to the experimental drought treatment. Increases in soil fungal diversity and large changes in functional group composition are observed in response to drought treatment within the amplitude of naturally occurring variation in soil water status. The treatment induces a conspicuous increase in diversity and abundance of dark-septate fungi (DSF), likely related to water regulation under moisture stress, and in the reduction of host susceptibility to soil-borne pathogens, whose richness decreases significantly in the experimentally droughted forest soil. The high seasonality of EEA in the control and its substantial suppression in the drought treatment, together with an increased N demand under experimental drought in the dry season suggest that the changed soil microbiota activity may signal a pending shift in biogeochemical functioning of the forest, from predominately P limited, as commonly observed in Amazon lowland rain forests[47], towards N limitation.

Results

Fungal communities under seasonal and experimental drought. Differences in fungal community structure were related to both seasonality and drought treatment, resulting in four distinct communities (Fig. 2; Supplementary Figs. 1, 2). Communities were dominated by Ascomycota, with mean relative

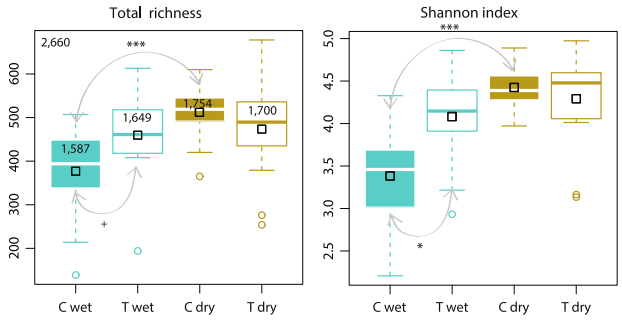

Fig. 3 Diversity of soil fungal communities in a lowland rain forest with respect to precipitation seasonality and in response to experimental drought. Changes in total species richness and Shannon index of soil fungal communities in response to seasonality and 14 years of throughfall exclusion (~50%) in a tropical lowland rain forest in Amazonia. Green, wet season; brown, dry season; boxplots—filled, control; empty, treatment. Total richness (number of OTUs), upper left corner of panel; total number of OTUs in each treatment/season inside plotted boxes. Significant differences as determined by paired *t*-tests or Wilcoxon signed-rank tests, corrected for multiple comparisons using the false discovery rate method are indicated by grey arrows; $^{+}p < 0.08$ (marginally non-significant); $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Boxplots: centre line, median; empty square, mean value; box limits, 25th and 75th percentiles; whiskers, 1.5 times interquartile range; empty circles, outliers.

abundances (proportion of reads, RA) of 62.8% and mean species richness (SR), defined as total number of operational taxonomic units (OTUs), of 73.1%, followed by Basidiomycota (RA, 32.5%; SR, 17.5%), Mucoromycota (RA, 2.3%; SR, 0.94%) and Rozellomycota (RA, 0.4%; SR, 2.2%; Supplementary Fig. 3). Chytridiomycota, Entomophtoromycota, Glomeromycota and Mortielellomycota represented together 0.3% of RA and 0.61% of SR, while unidentified OTUs accounted for 1.7% of RA and 5.6% of SR.

Regarding seasonality, total fungal SR and the Shannon diversity index (*H'*) were higher in the dry season than in the wet season in the control forest (Fig. 3); both Ascomycota and Basidiomycota contributed significantly to these differences, having higher SR and RA values in the dry season than in the wet season (Supplementary Fig. 3). No differences were detected in the drought treatment in the seasonality of total fungal SR and *H'* (Fig. 3), or in SR and RA of phyla (Supplementary Fig. 3).

There was a large difference in community composition related to season, evident in both the control and drought treatments. PERMANOVA analyses made on the fungal community indicated significant compositional shifts between the rainy and dry seasons for both control ($F = 3.8$, $R^2 = 0.12$, $p < 0.001$) and treatment ($F = 1.8$, $R^2 = 0.07$, $p < 0.001$). Significant differences were also observed in the seasonality of saprotrophs, both for control ($F = 3.6$, $R^2 = 0.11$, $p < 0.001$) and treatment ($F = 1.7$, $R^2 = 0.07$, $p < 0.01$) and in the seasonality of DSF for control ($F = 3.1$, $R^2 = 0.1$, $p < 0.001$) with marginally non-significant differences for treatment ($F = 1.6$, $R^2 = 0.06$, $p = 0.08$). Differences in ß-diversity ($ß_{sor}$), composed of spatial turnover ($ß_{sim}$) and the nestedness of species assemblage ($ß_{sne}$), between seasons within treatments were $ß_{sim} = 0.52$ (equal to 85.2% of $ß_{sor}$) and $ß_{sne} = 0.09$ (14.8% of $ß_{sor}$) for the control vs. $ß_{sim} = 0.54$ (88.5% of $ß_{sor}$) and $ß_{sne} = 0.07$ (11.5% of $ß_{sor}$) for the treatment.

As with treatment, the values of total SR and *H'* were higher in the throughfall exclusion treatment than in the control in the rainy season (Fig. 3) and were mainly attributable to a significant increase in Ascomycota SR (Supplementary Fig. 3). Significant differences between control and treatment in the rainy season were also detected for RA of Ascomycota and Basidiomycota. Ascomycota showed higher values of RA in the treatment than in the control, while Basidiomycota showed the opposite. No differences in total SR and *H'* between control and treatment were detected in the dry season (Fig. 3). However, significant differences were detected in Basidiomycota SR, with lower values in the drought treatment than in the control (Supplementary Fig. 3). The RA of Ascomycota and Basidiomycota also differed significantly between-treatment and control in the dry season, having the same patterns as observed in the rainy season.

The fungal communities in the drought treatment were distinctly different from those in the control, in both the rainy season ($F = 7.3$, $R^2 = 0.23$, $p < 0.001$) and the dry season ($F = 8.2$, $R^2 = 0.24$, $p < 0.001$). All main functional groups contributed to these changes with significant differences between control and the drought treatment, both in the rainy season (saprotrophs: $F = 6$, $R^2 = 0.19$, $p < 0.001$; pathotrophs: $F = 5.2$, $R^2 = 0.17$, $p < 0.001$; DSF: $F = 7.2$, $R^2 = 0.22$, $p < 0.001$) and in the dry season (saprotrophs: $F = 8.1$, $R^2 = 0.24$, $p < 0.001$; pathotrophs: $F = 7.1$, $R^2 = 0.21$, $p < 0.001$; DSF: $F = 8.2$, $R^2 = 0.24$, $p < 0.001$). Beta-diversity measures between control and treatment were $ß_{sim} = 0.65$ and $ß_{sne} = 0.06$ in the rainy season and $ß_{sim} = 0.63$ and $ß_{sne} = 0.04$ in the dry season, with spatial turnover representing 92 to 94% of community dissimilarities ($ß_{sor}$).

Functional groups and drought. Of the total number of OTUs assigned to individual functional groups (41.6%), saprotrophs contributed 60.6% (809 OTUs), pathotrophs 9.6% (128 OTUs), DSF 7.5% (100 OTUs) and symbiotrophs 3.1% (42 OTUs).

Regarding seasonality, in the control, the total SR of saprotrophs was significantly higher in the dry season than in the rainy season; RA showed the opposite pattern (Fig. 4; Supplementary Fig. 4; Supplementary Note 1). In the treatment, the contribution of saprotrophs to total SR was higher in the rainy than in the dry season. No seasonal differences were detected for SR and RA in pathotrophs. Between-season differences within control included higher DSF total and higher relative SR and RA in the dry season than in the rainy season (Fig. 4). In the drought treatment, higher DSF relative SR was detected in the dry season when compared to the rainy season. No significant between-season differences were detected in symbiotroph total and relative SR, and in RA.

As with treatment, in the dry season the total SR of saprotrophs was lower in the drought treatment than in the control (Fig. 4; Supplementary Fig. 4; Supplementary Note 1). Total and relative SR of fungal pathotrophs were significantly lower in the drought treatment than in the control for both the rainy and dry seasons. Total SR and RA of DSF were higher in the treatment than in the control in the rainy season (Fig. 4). The relative SR of DSF was higher in the drought treatment than in the control in the dry season. No significant between-treatment differences were detected in symbiotroph total and relative SR and in RA.

Soil extracellular enzyme activities under drought conditions. Regarding seasonality, all enzymes in the control samples, except for Laccase (Lac), showed strong seasonal variations in their activities, having lower values in the dry than the rainy season (Fig. 5). For the control, the ratio of ß-Glucosidase:N-Acetylglucosaminidase (Gls:NAG) was higher in the dry season than in the rainy season while that of NAG:Acid phosphatase (Pho) showed an opposite pattern. Conversely, none of the enzyme activities in the treatment were related to rainfall seasonality.

As with treatment, in the rainy season, in the treatment, the activities of ß-Glucuronidase (Glr), and of Lac (the latter also in the dry season) were lower than in the control (Fig. 5). In the dry season, the activities of NAG and ß-Xylosidase (Xyl) were

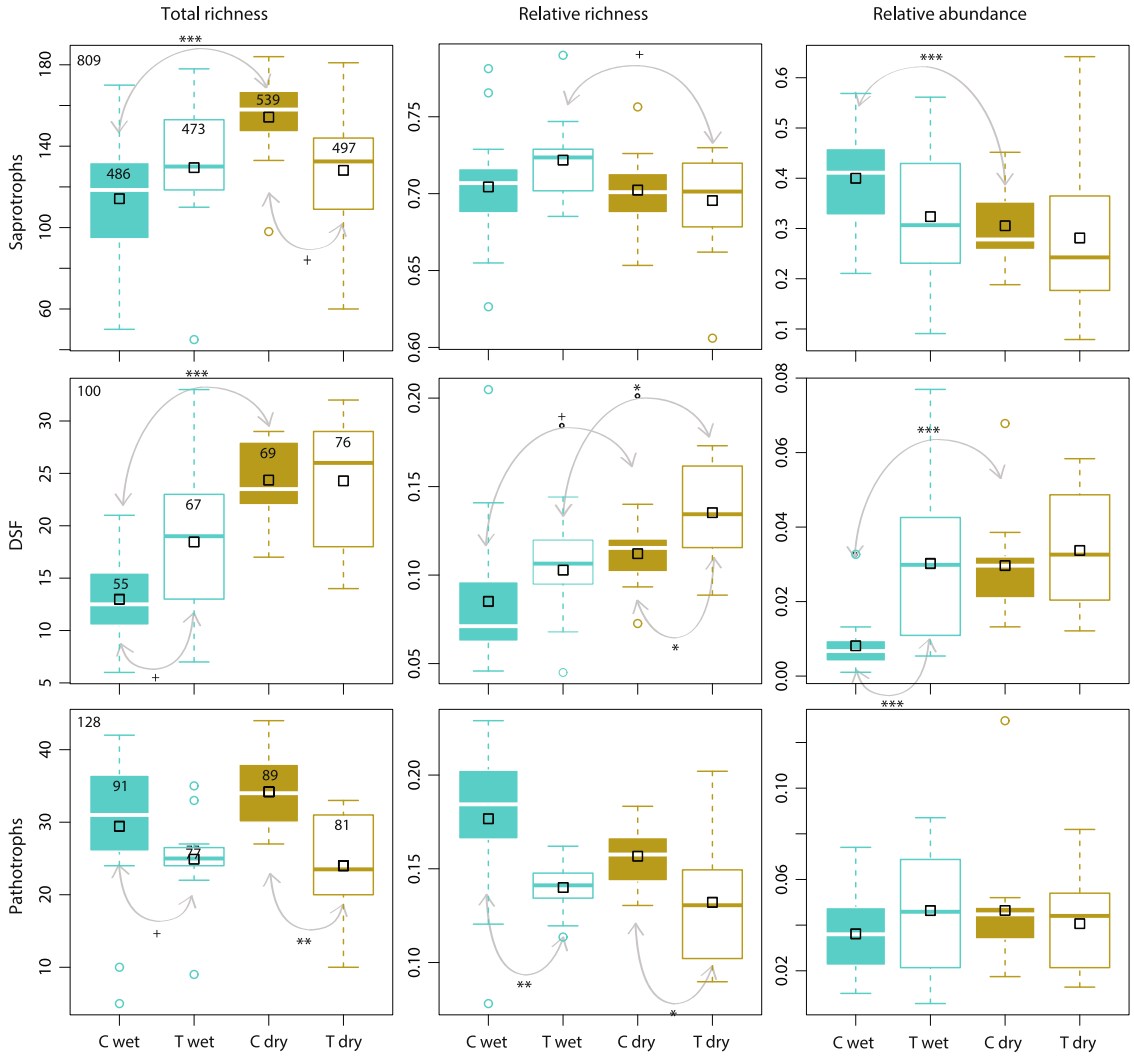

Fig. 4 Species richness and relative abundance of soil fungal functional groups in response to precipitation seasonality and experimental drought. Changes in species richness and relative abundance of the main soil fungal functional groups in response to seasonality and 14 years of throughfall exclusion (~50%) in a tropical lowland rain forest in Amazonia. Green, wet season; brown, dry season; boxplots—filled, control; empty, treatment. Total richness, number of OTUs in each functional group; total number for all samples per functional group in upper left corner of panels; total number per treatment per season inside plotted boxes; relative species richness, the proportion of OTUs assigned to a functional group relative to all OTUs assigned to functional groups; relative abundance, the proportion of reads. For total and relative species richness the complete pool assigned to a functional group consisted of 1335 OTUs. Significant differences as determined by paired *t*-tests or Wilcoxon signed-rank tests, corrected for multiple comparisons using the false discovery rate method are indicated by grey arrows; $^{+}p < 0.08$ (marginally non-significant); $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Boxplots: centre line, median; empty square, mean value; box limits, 25th and 75th percentiles; whiskers, 1.5 times interquartile range; empty circles, outliers.

significantly higher in the treatment than in the control. The ratio of Gls:NAG was lower in the treatment than in the control in the dry season, while the NAG:Pho ratio was higher in the treatment than in the control.

Soil attributes under seasonal and experimental drought. Regarding seasonality, between-season differences within treatments included higher pH, calcium (Ca^{2+}), potassium (K^{+}), magnesium (Mg^{2+}), sum of exchangeable bases (SB), base saturation of cation exchange capacity (V) and fine root biomass and lower coarse root biomass in the wet season for the control and higher soil moisture content in the wet season for both control and treatment (Supplementary Fig. 5).

As with treatment, in the rainy season, the treatment had lower concentrations of Ca^{2+}, phosphate (PO_4^{3-}), V and soil moisture content than the control. In the dry season, pH, Ca^{2+}, Mg^{2+}, nitrate (NO_3^{-}), SB and V were significantly lower and PO_4^{3-} and soil moisture higher in the control than in the treatment (Supplementary Fig. 5).

Discussion

We found that responses to natural seasonal drought in fungal community richness/composition, functional groups and soil extracellular enzyme activity (EEA) contrasted with those elicited by extreme drought. There was a high richness of dark-septate fungi (DSF), a fungal group that has not been reported quantitatively before in a tropical evergreen rain forest[48]. The richness of DSF was significantly higher in the dry season in the control and more so during the dry season in the drought treatment. This group was also the only functional group that responded with increased richness to extreme drought, indicating its potential involvement in alleviating the effects of soil moisture deficit (see below). The observed changes in EEA ratios indicated a potential switch from microbial P and C limitation under natural seasonal

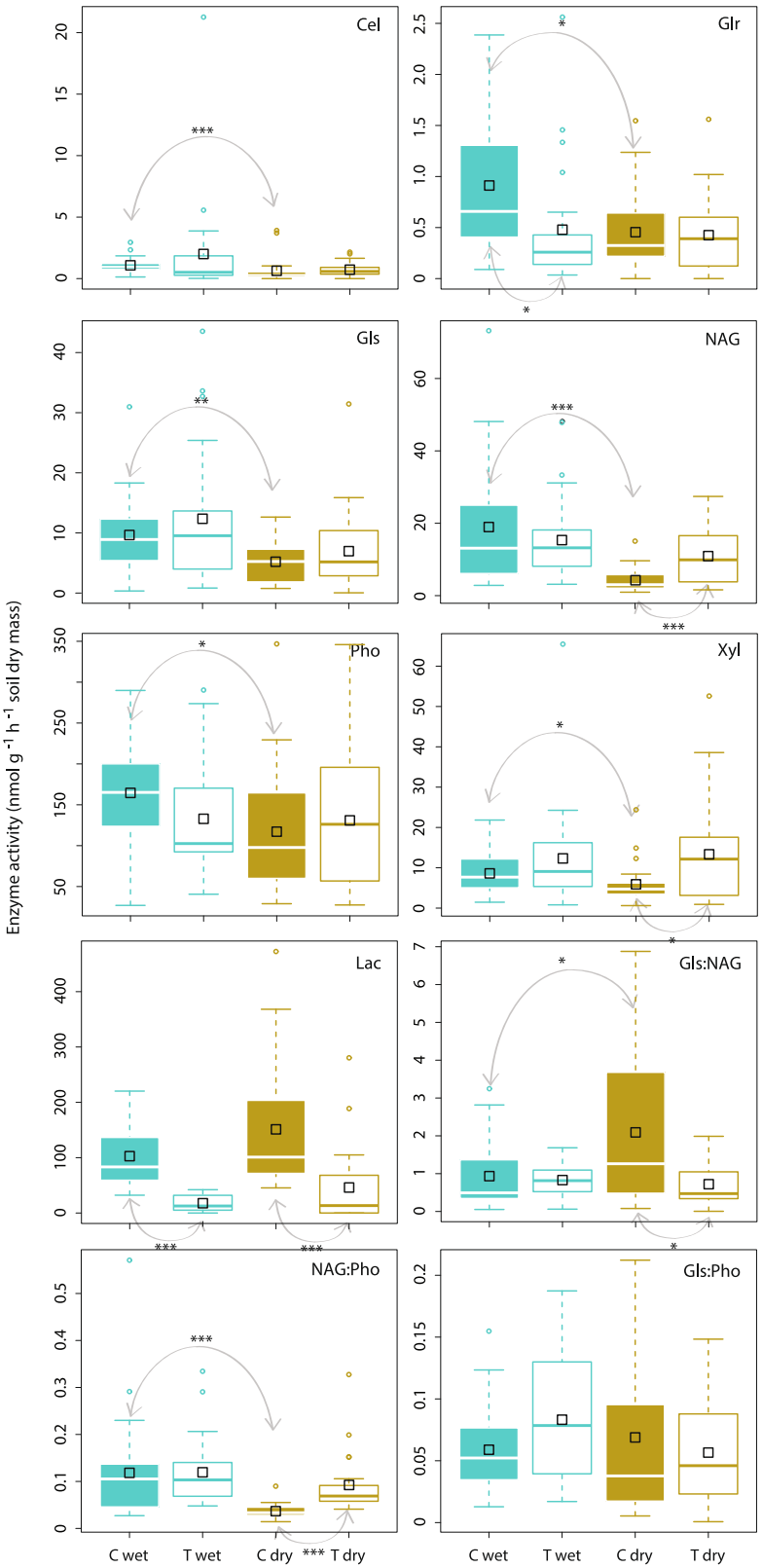

Fig. 5 Soil extracellular enzyme activity in response to precipitation seasonality and experimental drought in a lowland Amazonian rain forest. Changes in soil extracellular enzyme activity in response to seasonality and 14 years of throughfall exclusion (~50%) in a tropical lowland rain forest in Amazonia. Green, wet season; brown, dry season; boxplots—filled, control; empty, treatment. Cel Cellobiohydrolase, Glr ß-Glucuronidase, Gls ß-Glucosidase, Lac Laccase, NAG N-Acetylglucosaminidase, Pho Acid phosphatase, Xyl ß-Xylosidase. Significant differences as determined by paired *t*-tests or Wilcoxon signed-rank tests, corrected for multiple comparisons using the false discovery rate method are indicated by grey arrows; $^{+}p < 0.08$ (marginally non-significant); $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Boxplots: centre line, median; empty square, mean value; box limits, 25th and 75th percentiles; whiskers, 1.5 times interquartile range; empty circles, outliers.

drought, to incipient N limitation under long-term soil drought in the dry season. Significant seasonal differences in EEA were not observed within the drought treatment, suggesting that under long-term experimental drought, the additional moisture stress experienced during the dry season represents soil moisture conditions that are not amenable to further adjustment in EEA, i.e. the system may have reached its limits in terms of plastic and adaptive EEA functional capacity.

Fungal community structure was related to both seasonality and drought treatment, resulting in four distinct communities. Beta-diversity was manifest in large species turnover—between control and treatment, and between seasons within treatments—representing 85–94% of community dissimilarities; differences between control and drought treatment in both the rainy and dry seasons were highly significant. Precipitation seasonality resulted in significant compositional shifts for both control and treatment. Our hypothesis (H_{1A}) that the dry season in the control, representing the amplitude of naturally occurring variation in soil water status, would be associated with an increase in fungal taxonomic diversity was supported and to a much greater extent than has been observed for soil fungal richness and community composition in tropical rain forests. By limiting connectivity among soil pores, drought can potentially reduce competitive interactions among microbial taxa and increase microbial richness[49,50], a process that is likely to be enhanced in the relatively sandy soil at our study site. Additionally, reduced connectivity of the pore space is likely to favour filamentous fungi vs. unicellular taxa[51]. This was borne out in our study whereby yeasts showed a consistently decreased abundance both in the dry season and in the treatment. Seasonal variation in the hydrological balance of tropical lowland rain forests is expected to result in adaptations in soil fungal communities[38,52] that might temper their responses to precipitation manipulation treatments. Adaptation and acclimation of individual microbial taxa have been cited as explanations for why climate manipulations have previously had no effect on fungal community composition over the long term[53]. We show here that a large and long-term reduction of precipitation combined with a sandy soil texture has re-shaped the fungal community over a period of 14 years at our study site, resulting in a major shift in the suite, and relative abundances, of the species recorded.

Beyond compositional change, the experimental drought in the dry season not only did not reduce fungal taxonomic diversity, but maintained instead the same diversity levels found within the amplitude of naturally occurring soil drought conditions. This result therefore contradicted the expectations of part of our hypothesis (H_{1B}) regarding the effects of a large long-term press disturbance. The same pattern was observed in the seasonality of the treatment forest, with no differences in taxonomic diversity between the rainy and dry seasons despite the substantial seasonal change in the overall community. The contrast in community composition between control and treatment, despite the reported ability of fungi to withstand very low soil water potential (p. 52, ref. [54]) may be expected after 14 years of partial throughfall exclusion. Although the control and treatment plots are within a distance of 50 m, the resulting contrast in soil moisture values are typical of differences between biomes separated by several thousand kilometers. Therefore both meta-community (ecological scale) and biogeographical approaches are relevant in interpreting the changes in fungal community structure and the relative contribution of taxa to soil biogeochemical processes[41]. Following the meta-community approach, a combination of species filtering and patch dynamics may be the most likely mechanisms that have led to contrasting communities between control and treatment. Species sorting assumes no restriction in dispersal. In our case, there is an asymmetrical dispersal and exchange of input of propagules between the control and the treatment plots. Both plots receive an unquantified amount of input of fungal propagules from the surrounding forest matrix, and species sorting occurs at the treatment plot level. However, the input via dispersal from the treatment plot to the control plot, given the size (and expected relative contribution) of the treatment plot in relation to the surrounding matrix must be rather small, and this may further have contributed to the differences arising from species sorting. Additionally, patch dynamics may have further amplified the differences. Patch dynamics implies a trade-off between dispersal capacity and competitive strength among species[55,56]. In our experiment, with reduced throughfall and the death of a number of large trees—which have modified the availability of soil water and organic C and other elements—it is likely that the conditions enhancing such patch dynamics effects (competitive advantage of taxa better suited to the droughted soil environment) would be manifest. To what extent species sorting, resulting from regional and occasional long-distance dispersal, has contributed to contrasting patterns is less clear. Nonetheless, it is possible that the priority effect of the existing community has changed over time with the exclusion of throughfall, and the probability for the contribution of input from incidental long-distance dispersal from regional and intercontinental[57–59] airborne propagules may have increased. While allochthonous microorganisms may easily be outcompeted on arrival in natural ecosystems[60], severely impacted environments such as our drought treatment are likely to act as a novel environmental filter and open up ecological niches where the 'priority effect' of the resident microbial community may no longer hold and allochthonous microbes may successfully establish and colonise. Finally, we hypothesise, but cannot ascertain, the potential contribution of rapid evolution and ecological adaptation to prevailing soil conditions, as has been demonstrated for heritable genetic modification in soil bacteria and fungi[61,62].

The variation in fungal communities within functional groups was related mostly to treatment and to a lesser extent to seasonality. Increasing drought led to an increase in the richness and abundance of DSF, with highest diversity in the extreme drought conditions. These fungi, which exhibit little if any host specificity[48], have often been reported in stressful and nutrient-limited environments, including polar and alpine habitats[63], but hardly ever in tropical forests[48]. They are likely to be involved in increasing host tolerance to stressful environmental conditions, such as soil water shortage, owing to their ample production of melanised tissues that confer rigidity to their cell walls and protect them from desiccation and radiation[64]. An extensive colonisation by DSF of host plants has been suggested to be an indication of altered water conductance within host tissue; DSF have been hypothesized to be involved in the alteration of root water dynamics under stressful environmental conditions by modifying the resistance to flow in roots as well as by being involved in the alteration of plant transpiration rates and stomatal conductance[65]. Such changes in water use[66] and hydraulic resistance and conductivity in trees[20] have been observed at our study site. Since plant species respond differently to colonisation by DSF, changes in DSF diversity and composition, especially changes in abundance of those taxa that have a broad host range and differ in their impacts on host performance, could have an impact on plant community composition in the long term[67]. At our site, there has been a switch reported from an early reduction in transpiration rates per tree in the first two years of the drought treatment[68], to higher transpiration rates per tree following long-term mortality (i.e. leaving fewer trees) in the drought treatment[66], which might be associated with a build-up of a DSF effect over time.

DSF are also likely to have a role in reducing host susceptibility to soil-borne pathogens and in facilitating nutrient uptake by host

plants, especially from recalcitrant and complex organic sources[69,70]. The highest DSF species richness and abundance in the drought treatment (both seasons) at our study site were accompanied by the lowest pathogen richness values. Mandyam and Jumpponen[65] have suggested that DSF might inhibit pathogens or minimise their impacts through: (i) direct competition for plant photosynthates and/or sites of colonization; (ii) production of inhibitory compounds and (iii) by inducing plant defense responses to pathogen infection. A decrease in pathogen richness driven by prolonged drought events (i.e. consistent with future climate scenarios[71]), could also be responsible for shifts in tropical forest communities as predicted by the Janzen–Connell hypothesis (JCH)[72,73]. The JCH proposes that specialist natural enemies maintain diversity in plant communities by reducing the survival rates of conspecific propagules located close to reproductive adults, or in areas of high conspecific density. A recent meta-analysis of studies that tested the JCH identified a trend for stronger distance- and density-dependence in wetter sites than in sites with lower annual precipitation[74]. The results reported here support the predictions made by the JCH and suggest that regardless of the specific mechanisms involved (i.e. biotic interaction between plants and their natural specific enemies and/or intraspecific competition), predicted future shifts in precipitation patterns due to anthropogenic climate change[71], could alter biotic interactions that underlie distance- and density-dependence, with ensuing consequences for species composition and diversity in ecological communities.

The large change we observed in fungal diversity in response to long-term drought is of interest in itself; however, the impacts that it may have on nutrient cycles is of fundamental relevance for projecting future changes on ecosystem functioning. Rain forests are generally marked by seasonal variation in nutrient availability[46,75], associated with crashes in soil microbial populations following desiccation[76] and in all measures of microbial biomass[77] and activities, such as litter decomposition[78,79], nitrification/denitrification[16,80] and respiration[81,82], as also observed at this site[24]. EEA is likely to be affected by a combination of non-mutually exclusive mechanisms such as shifts in microbial community composition and related functional traits, and changes in biotic interactions, and soil nutrient availability[83–85]. Drought conditions may also impose diffusion limitations on enzyme activity and substrate availability, as well as the metabolic uptake of the products of EEA[86]. Our results on EEA values (expressed on a g^{-1} SOM basis) are comparable to those reported for neotropical lowland forests[30,32,33,87]. Additionally, our high rates in the control of Pho compared to enzymes involved in C (Gls) and N (NAG) acquisition (i.e. ratios of Gls:Pho and NAG:Pho < 0.1), were very similar to those found in a rain forest in Panama[32] and point to a strong P limitation.

As with seasonality, the observed pattern of EEA in the non-droughted control forest supported our expectation of a reduction in enzymatic activity being related to precipitation seasonality (H_2), in line with the very few studies that have been reported for other tropical rain forest soils[29,32]. In the control, all enzymes showed strong reductions in their activities in the dry season, with the exception of Lac. The ratio of NAG to Pho was 3-fold smaller in the dry season while the ratio of Gls to NAG showed a 2.4-fold increase. These EEA ratios indicate in the control that P and C acquisition are being favoured relative to that of N during the dry season. The decline in potential activities of N-acquiring enzymes relative to P- and C-acquiring ones indicates a reduction of organic N degradation in conditions of reduced soil moisture, and could possibly be due to the increase in available soil mineral N associated with soil microbial crashes following desiccation in the dry season[46].

The EEA in the drought treatment did not support our hypothesis (H_2) that predicted a significant reduction in enzyme activities in the dry season. The absence of significant seasonal changes in EEA in the drought treatment contrasts with the marked seasonal shift in the control and may signal an incipient change in biogeochemical functioning in the forest. Few enzymes related to the C and N cycles responded to the drought treatment, which also did not affect the activity of Pho (P cycle). The hypothesised reduction in EEA induced by the treatment relative to the control (both in the rainy season and more strongly in the dry season) was therefore not supported by the results. While nutrient availability has been shown to stimulate the activity of hydrolytic enzymes, oxidative enzymes are instead likely to be activated by water availability[88,89]. This appeared to be the case at our site, where the potential activity of Lac was lower in the treatment than in the control in both the rainy and dry seasons. Regarding the N cycle, in the dry season, the activity of NAG, involved in the autolysis of senescing or dead mycelium and insect exoskeleton tissue for recycling N[90] was significantly higher in the treatment than in the control (we observed greater presence of termites in the throughfall exclusion treatment than in the control). In addition to being responsible for the degradation and hydrolysis of chitin, chitinase and chitinolytic enzymes are also considered as the major structural component of many fungal cells that use hyper-parasitism against pest and pathogen attack[91]. Chitinase production can be induced and can accumulate in response to microbial infections, and it is thought to be involved in the defense of plants against pathogen infections[92]. The higher potential activity of NAG in the treatment in both seasons could therefore also be linked to the observed decrease of pathogens in the treatment.

In the drought treatment, higher NAG to Pho ratios (2.3-fold greater) and lower Gls to NAG ratios (1.9-fold less) in the dry season contrasted with those observed in the control. These EEA ratios in the treatment in the dry season indicate a putative increased N demand. Critically, we did not observe significant between-season differences in EEA in the drought treatment which indicates that during the dry season in the drought treatment, soil moisture conditions are not amenable to further adjustment in EEA, i.e. soil EEA in the drought treatment has reached its limits in terms of plastic and adaptive functional capacity. It has been shown that when environmental changes exceed the range of adaptation by communities, large ecosystem-level shifts can occur, with direct impacts on ecosystem processes (e.g. CO_2 efflux;[41]). For example, rhizosphere respiration at the study site has been reduced in the treatment, while soil heterotrophic respiration, a widely used proxy of decomposition, has increased[17], an outcome also reported worldwide across ecosystems[93].

In summary, our experimental results provide evidence that projected increases in drought frequency, duration and severity in tropical rain forests could indeed modify C, N and P cycles, with consequences for other ecosystem processes. The long-term experimental exclusion of throughfall in a tropical evergreen rain forest has indicated the highly likely involvement of DSF in alleviating the effects of drought on trees. This is likely to have occurred through compensatory mechanisms that improved nutrient and water acquisition by trees in the droughted forest, and therefore contributed to maintaining ecosystem functioning during the long-term drought. After 14 years of throughfall exclusion, this forest appears to have progressed towards an alternative edaphic state where key biogeochemical processes appear to be altering (Fig. 6). Throughfall exclusion appears to have induced changes in nutrient cycling by shifting P and C limitation of microbial communities under natural precipitation seasonality, to incipient N limitation following experimental long-term soil drought that is superimposed upon naturally low precipitation during the dry season. The high seasonality of the EEA

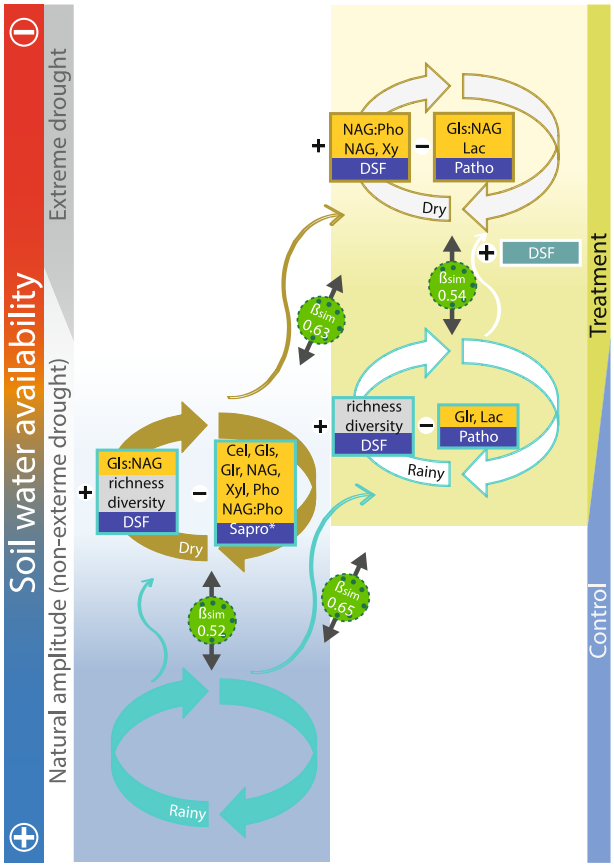

Fig. 6 Seasonal and experimental drought effects on microbial nutrient cycling and fungal communities. Changes in soil extracellular enzyme activities (EEA) and ratios, fungal community richness, diversity and turnover, and main fungal functional groups, in response to seasonal (dry vs. rainy) and experimental drought (14 years of throughfall exclusion, ~50%) in a tropical lowland rain forest in Amazonia. Long-term throughfall exclusion induced changes in nutrient cycling by shifting P and C limitation of microbial communities under natural precipitation seasonality (light blue panel), to incipient N limitation under long-term soil drought combined with seasonal negative hydrological balance (light green panel; for details on EEA see Fig. 5). Drought induced a significant increase of dark-septate fungal richness and relative abundance in the natural range of moisture amplitude and an even more accentuated increase in richness under extreme drought conditions (i.e. treatment × dry season; see also Fig. 4), and a decrease of pathogens richness in experimental drought conditions in both seasons. Drought also induced the increase of fungal richness and diversity in the natural range of moisture amplitude (see also Fig. 3). ±, statistically significant difference between entities linked by wavy arrow; green, wet season; brown, dry season; filled, control; empty, treatment; blue, fungal functional groups; DSF dark-septate fungi, Patho pathogens, Sapro* saprotrophs relative abundance (note that saprotroph richness was inversely related to abundance), yellow, soil extracellular enzyme activity and ratios; Cel Cellobiohydrolase, Gls ß-Glucosidase, Glr ß-Glucuronidase, Lac Laccase, NAG N-Acetylglucosaminidase, Pho Acid phosphatase, Xyl ß-Xylosidase.

in the control, and the remarkable suppression of such seasonality in the treatment are probably an indication of the system operating at its limits in terms of functional adaptability under long-term soil drought. The clear lack of seasonality of diversity of all functional groups except for DSF and of all soil variables (except for moisture content) also strongly supports the contention that the soil component of the ecosystem has reached limitations in its functional adaptability under long-term soil drought. Our findings suggest that future projected climate change could, in addition to the significant tree- and ecosystem-level impacts demonstrated in earlier work[15], substantially affect soil microbial communities, functional groups and wider biogeochemical cycles, with further effects on the functioning of these ecosystems and their continued provision of essential ecosystem services.

Methods

Study area. The site lies *ca.* 350 km to the west of Belém, within the Caxiuanã National Forest Reserve (1°43'S, 51°27'W), Pará state, eastern Amazonia, Brazil. The mean elevation is at 20–25 m a.s.l., and the area is characterised by an equatorial climate, tempered by large bodies of open water nearby. The mean annual temperature is 25 °C and mean annual precipitation is between 2000 and 2500 mm[23], with a pronounced drop in precipitation between June and November (monthly rainfall < 100 mm). The soil is a yellow oxisol developed over a deep lateritic profile with a proportion of sand at 0–0.5 m of 75–83%[94]. The vegetation is lowland evergreen rain forest, free of apparent recent disturbances, however, in the soil, frequent charcoal fragments have been observed to a depth of 30 cm (L. Nagy pers. obs.).

Experimental treatment and sampling design. A rainfall exclusion experiment was established in 2001, where *ca.* 50% of the canopy throughfall was excluded from the soil of a 1 ha (100 × 100 m) plot using polythene panels supported at *ca.* 2 m above ground level[15,68]. A paired control plot of equal dimensions was delimited at *ca.* 50 m apart from the throughfall exclusion treatment plot. Trenching to a depth of 1–2 m along the perimeters of both plots was applied to control lateral superficial and sub-superficial water flow from outside of the plots. Each of the plots were subdivided into 25 subplots of 20 × 20 m. Analyses of the experimental approach and the effects of this soil drought treatment on tree, soil and ecosystem functioning can be found in refs. [15,35].

Soil sampling. For soil sampling coordinates for random sampling points were generated for each subplot by the 'Sample' module in TerrSet[95]. A peripheral buffer zone of 10 m inside the 100 × 100 m plots was excluded from sampling. Soil samples were collected in 2016 during two field campaigns, one in the rainy (15–25 Feb) and one in the dry season (17–27 Oct). The samples collected in the control (wet and dry seasons) and in the throughfall exclusion treatment in the wet season fell within the natural range of observed seasonal variability in soil moisture (non-extreme drought), while those collected in the throughfall exclusion treatment in the dry season fell outside of this range (extreme drought; Fig. 1). Within 1 m distance of each sampling point and after removing the thin litter layer, three soil samples were extracted, to a depth of 5 cm, using a soil corer of 5 cm diameter. The three samples were pooled into a composite sample per sampling point, resulting in a total of 100 samples (2 sampling dates × 25 points for both control and treatment). Soil samples were stored in polythene bags and kept in thermal insulated boxes cooled by ice gel packs. On arrival in the laboratory, subsamples for molecular analyses were separated and stored in 2.5 ml Eppendorf vials at −20 °C; for enzymatic assays and the determination of mineral nitrogen (N) and root biomass, the samples were stored in polythene bags at 4 °C, while for all the other soil chemical analyses subsamples were air dried (Supplementary Methods 1).

Molecular analyses and fungal sequence processing. DNA extractions were obtained with a PowerSoil DNA isolation Kit (MoBio Laboratories) by using 0.25 g of soil. Amplicon libraries were prepared by PCR amplification of the variable region of the fungal internal transcribed spacer 2 (ITS2) with the primers fITS7[96] and ITS4[97]. The molecular work was carried out at the Naturalis Biodiversity Center, while the Illumina MiSeq sequencing was done at BaseClear in Leiden, The Netherlands. Sequencing was successful for a total of 93 samples. After the exclusion of singletons, the dataset contained 905,322 sequences in 7284 OTUs (deposited at DDBJ/EMBL/GenBank under the BioProject accession number PRJNA579137). We followed then the conservative approach suggested by Lindahl et al.[98] and further removed all OTUs with less than five occurrences across all samples and less than five sequence counts in a sample. The dataset for the final analyses consisted of 884,105 sequences (2660 OTUs), with an average of 9507 (range: 921–33,286) reads per sample. Sequence assignment to taxonomic groups was based on pair-wise similarity searches against the UNITE fungal ITS sequence dynamic SH database[99]. OTUs were assigned to 'functional groups' (i.e. trophic groups) by searching against the FUNGuild database[100]. Rarefaction accumulation curves were calculated for each sample 'vegan' R package;[101] to explore the completeness of our sampling. The sequencing depth of all samples was high and rarefaction curves tended to reach a saturation point for all samples (Supplementary Fig. 6).

Enzyme assays. The extracellular enzyme activity (EEA) of eight hydrolytic and oxidative enzymes (N-Acetylglucosaminidase, NAG; Cellobiohydrolase, Cel; ß-Glucosidase, Gls; ß-Glucuronidase, Glr; Laccase, Lac; Acid phosphatase, Pho and ß-Xylosidase, Xyl) was determined by following the procedure described by ref. [102]. Fluorescence and absorbance intensities were measured with a computerized

microplate fluorimeter and colorimeter (Biotek, Synergy 2). Fluorescence measurements were carried out at 368-nm excitation and 465-nm emission. Absorbance measurements for diammonium 2,2'azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) were made at 425 nm. For detailed description on the preparation of calibration solutions, buffers and substrates and on the determination of the profiling of soil EEA see Supplementary Methods 2[103].

Data treatment and analyses. Spatial autocorrelation of fungal community composition at the sample level was determined for each of the control and treatment plots in both seasons. Geographical distances were assessed by Mantel correlogram, using the Pearson's correlation method with 9999 permutations ('vegan' R package). The progressive Holm method was used to correct significant values across distance classes for multiple comparisons. Subsequent analyses on communities were conducted on non-rarefied data unless specified, on exclusively spatially non-autocorrelated and therefore independent samples. The number of independent samples was 55 and the number of OTUs included in the analyses was 2660. Of these, 256 were assigned to multiple functional groups and therefore excluded from further analyses; 1079 were assigned to individual functional groups and were used to for calculations of representation of functional groups. This naturally may have biased the resulting proportions for functional groups. Spatial and temporal variability in species composition were determined by calculating ß-diversity, including its partitioning into spatial turnover ($ß_{sim}$) and nestedness of species assemblage ($ß_{sne}$) between control and treatment and between seasons within treatments and were computed R package 'betapart' v.1.3[104]. Seasonal and treatment-induced shifts in soil fungal community composition were visualised with non-metric dimensional scaling (NMDS) on rarefied and non-rarefied data, based on the Bray–Curtis dissimilarity measure and carried out on both presence/absence and relative abundance data. An ordination of community structure performed with the complete set of samples indicated no significant effect of removing spatially autocorrelated samples (Supplementary Fig. 7). Changes in community composition between control and treatment with season were assessed on non-rarefied presence/absence and relative abundance data with the ADONIS function in 'vegan' with 10,000 permutations. To be able to analyse the differences in functional groups between control and drought treatment and between seasons, NMDSs were run separately for each of the most representative functional groups, on presence/absence and relative abundance. Total fungal OTUs species richness (SR) and Shannon index (*H'*), and the total/relative SR and relative abundance (RA) of the most representative functional groups were calculated to assess the differences between treatments and between seasons within treatment. The 'envit' R function was used to fit these parameters together with soil variables and EEA onto the NMDS ordinations after exclusion of highly correlated soil variables (e.g. Ca^{2+}, K^{+} and CEC).

There are several fungal species that form both ericoid- (ERM) and dark-septate fungi (DSF)-like structures on different hosts as well as on the same host, suggesting that the taxonomical and morphological divide between DSF and ERM association is not clear-cut[105,106]. We considered this group that was classified as ERM in the 'guild' section and as DSF in the 'growth morphology' section by searching against the FUNGuild database, as DSF. The choice was guided by the lack of ericoid species in lowland evergreen rain forests and also because the reference cited in FUNGuild[100] for all these OTUs refers exclusively to DSF.

The effects of treatment and seasonality on diversity indices, non-rarefied RA of fungal taxonomic and functional groups, SR and relative SR, EEA and soil properties were assessed with t-test or Wilcoxon test for non-normally distributed data. Statistical significance was corrected for multiple comparisons using the false discovery rate (FDR) method[107].

Data availability

The dataset contained 905,322 sequences in 7284 OTUs that were deposited at DDBJ/EMBL/GenBank under the BioProject accession number PRJNA579137.

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Competing interests

The authors declare no competing interests.

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