Delayed wet season increases soil net N mineralization in a seasonally dry tropical forest

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Abstract

Seasonal precipitation regime plays a vital role in regulating nutrient dynamics in seasonally dry tropical forests. Present evidence suggests that not only wet season precipitation is increasing in the tropics of South China, but also that the wet season is occurring later. However, it is unclear how nutrient dynamics will respond to the projected precipitation regime changes. We assessed the impacts of altered seasonal precipitation on soil net N mineralization in a secondary tropical forest. Since 2013, by reducing throughfall and/or irrigating experimental plots, we delayed the wet season by two months from April - September to June - November (DW treatment) or increased mean annual precipitation by 25% in July and August (WW treatment). We measured soil net N mineralization rates and assessed soil microbial communities in January, April, August and November in 2015 and 2017. We found that a wetter wet season did not significantly affect soil microbes or net N mineralization rates, even in the mid-wet season (August) when soil water content in the WW treatment increased significantly. By contrast, a delayed wet season enhanced soil microbial biomass and altered microbial community structure, resulting in a two-fold increase in net N mineralization rates relative to controls in the early dry season (November). Structural equation modeling showed that the changes in net N mineralization during the early dry season were associated with altered soil microbial communities, dissolved organic N, and litterfall, which were all affected by enhanced soil water content. Our findings suggest that a delayed wet season could have a greater impact on N dynamics than increased precipitation during the wet season. Changes in the seasonal timing of rainfall might therefore influence the functioning of seasonally dry tropical forests.

Keywords: precipitation change, N mineralization, soil microbe, climate change

1 Introduction

Altered precipitation regimes are an important aspect of global change (IPCC, 2013), as they can substantially affect a wide range of terrestrial ecosystem processes (Cusack et al., 2016; Allen et al., 2017). Precipitation change is forecasted to manifest as larger rainfall events and longer intervals between events, which alters soil moisture regimes (Weltzin et al., 2003; Seneviratne et al., 2010) and may result in more frequent or more severe soil water deficit in dry ecosystems (Knapp et al. 2008). Seasonally dry tropical forests are characterized by low annual rainfall and distinct dry seasons with <100 mm of rainfall per month (Allen et al. 2017). The ecological structure and functions of seasonally dry tropical forests are therefore strongly regulated by the temporal pattern of precipitation (Jaramillo et al., 2011; Allen et al., 2017). Seasonally dry tropical forests in South China may be particularly strongly affected by precipitation change, as they are alternately influenced by oceanic and continental monsoons (Dirzo et al., 2011) and climate models forecast both a later wet season and increased precipitation during the wet season across the region (Fang et al., 2004; Luo et al., 2008; Zhou et al., 2011). However, we know little about how changes in the timing and amount of the wet season precipitation will affect ecosystem processes in these seasonally dry tropical forests.

The seasonality of rainfall results in nutrient pulses that are an important part of tropical forest nutrient cycles (Lodge et al., 1994). Precipitation change can thus have a major effect on ecosystem function by altering nutrient cycling (Yahdjian et al., 2006; Ollivier et al., 2011; Cregger et al., 2014; Song et al., 2020). Synchrony between nutrient availability and plant uptake is crucial for reducing losses of limiting nutrients in tropical forests (Lodge et al., 1994). In this context, the timing of N availability might be particularly important because N is an essential macronutrient for plants and soil organisms, inorganic forms of N are highly mobile in soils, and N is one of the elements limiting tropical forest productivity (Kaspari et al., 2008; Wright et al., 2011). However, multiple processes involved in soil N cycling are regulated by soil moisture (Robertson and Groffman, 2007) and are therefore likely to be affected by precipitation change (Chen et al., 2019). Soil net N mineralization (net N_{min}) is the balance of gross N mineralization and N immobilization by soil microbes, which are regulated by precipitation (Wang et al., 2006), temperature (Verburg et al., 1999), plant characteristics (Knops et al., 2002), soil properties (Carlyle and Nambiar, 2001) and microbial communities (Yokobe et al., 2018). Understanding how net N_{min} is affected by precipitation change is important because it can be a good indicator of soil quality (González-Prieto et al., 1992) and N availability for plants (Neill et al., 1999; Schimel and Bennett, 2004). However, experiments investigating the response of net N_{min} to precipitation changes are inconclusive, with some demonstrating that soil net N_{min} is sensitive to precipitation changes (Jamieson et al., 1998; Emmett et al., 2004; Zhu et al., 2017), whereas others have measured no response (Yahdjian and Sala, 2010; Cregger et al., 2014; Schaeffer et al., 2017). In addition, most studies investigating the effects of precipitation changes on net N_{min} simulated changes in annual precipitation amounts, and typically increased or reduced precipitation persistently throughout a year (Knapp et al., 2008; Beier et al., 2012), which does not account for projected shifts in the timing of rainfall events (IPCC, 2007, 2012). Importantly, despite the demonstrable importance of rainfall seasonality for N cycling in seasonally dry tropical forests (Singh et al., 1989; Davidson et al., 1993; Verma et al., 2013), we are unaware of any studies investigating the response of net N_{min} rates to changing precipitation patterns in seasonally tropical dry forests.

Soil net N_{min} is directly regulated by soil microbial metabolic activity and soil moisture is one of the most important abiotic controls of soil microbial community structure and activity (Wagener and Schimel, 1998; Bardgett et al., 1999; Fierer et al., 2003; Brockett et al., 2012; Manzoni et al., 2012). Hence, precipitation changes can substantially affect soil microbial biomass (Liu et al., 2009; Naylor and Coleman-Derr, 2018; Xu et al., 2020), community structure (Taylor et al., 2004; Sorensen et al., 2013; Li et al., 2017) and microbial guilds responsible for soil N transformations (Fuchslueger et al., 2014; Chen et al., 2017; Kaurin et al., 2018). The effect of precipitation change on net N_{min} therefore occurs indirectly via the influence of soil moisture on the microbial communities involved (Fierer and Schimel, 2002; Bengtsson et al., 2003; Smithwick et al., 2005; Ribbons et al., 2016). Net N_{min} rates are often directly related to the size of the soil microbial biomass (Bengtsson and Bergwall, 2000; Remy et al., 2018) and changes in soil microbial community structure could affect organic matter decomposition, microbial N demand, and thus inorganic N release (Bengtsson et al., 2003; Tiemann and Billings, 2011; Ribbons et al., 2016). In addition, changes in microbial guilds responsive for soil N transformation could directly alter net N_{min} rates (Leininger et al., 2006; Petersen et al., 2012; Levy-Booth et al., 2014). However, soil microbial communities may acclimate to variable soil moisture levels, thus maintaining their functions despite precipitation changes (Bell et al., 2014; Canarini et al., 2016). Moreover, as the physicochemical and biotic conditions differ temporally, microbial response to precipitation change could also vary among seasons or years (Gutknecht et al., 2012; Sorensen et al., 2013). Given that the effect of precipitation change on net N_{min} is overwhelmingly mediated by soil microbes, the soil microbial response to precipitation change should be carefully examined to learn the underlying mechanisms regulating the response of net N_{min} to precipitation changes.

We established a field precipitation manipulation experiment in a seasonally dry tropical forest to simulate projected changes in precipitation regimes (a delayed wet season and a wetter wet season) in the tropics of South China in 2012. In the present study, carried out between 2015 and 2017, we aimed to: (1) determine the response of net N_{min} rates to a delayed wet season and a wetter wet season; and (2) assess how soil microbial communities mediate the effects of the precipitation changes on net N_{min}. Our previous work demonstrated that a delayed wet season increased soil water content throughout the year, resulting in greater soil microbial biomass and litter decomposition rates, whereas the wetter wet season increased soil water content only during the mid-wet season, and did not affect soil microbial biomass or litter decomposition (Yu et al., 2019; 2020). As a result, we hypothesized that: 1) a delayed wet season would result in higher net N_{min} rates throughout the study period, whereas a wetter wet season would result in reduced net N_{min} rates in the mid-wet season, and 2) net N_{min} rates would be related to differences in soil microbial biomass and community structure among treatments.

2 Materials and Methods

2.1 Site description

The precipitation manipulation experiment was established in a 60-year old seasonally dry tropical forest (Ding et al., 1992) at the Xiaoliang Tropical Coastal Ecosystem Research Station (110°54′E, 21°27′N), Chinese Academy of Sciences, Guangdong Province, China. The climate is tropical with a mean annual temperature of 23℃ and mean annual precipitation of 1400-1700 mm. More than 70% of the precipitation falls during the wet season from April to September. The soil is classed as lateritic on deeply weathered granite. Based on a 2015 survey, the dominant canopy tree species at the site were *Aphanamixis polystachya*, *Schefflera octophylla*, *Carallia brachiate*, *Symplocos chunii*, *Acacia auriculaeformis*, *Photinia benthamiana* and *Cinnamomum burmanni*, and the dominant understory species were *Dicranopteris dichotoma*, *Lygodium japonicum*, *Blechnum orientale, Psychotria rubra*, *Uvaria microcarpa* and *Clerodendrum cyrtophyllum* (Yu et al. 2019).

2.2 Experimental design

2.2.1 Field study

In 2012, we established four replicate blocks comprising three 12-m × 12-m plots, spaced at least 3 m apart. The following three treatments were randomly assigned to one plot per block: 1) delayed wet season (DW), in which a combination of rainout shelters and irrigation effectively shifted the wet and subsequent dry season by *c.* two months, 2) wetter wet season (WW), in which we used irrigation to simulate a *c.* 25% increase in annual precipitation during the mid-wet season, and 3) a control (CT) with ambient precipitation inputs. In the DW and WW treatments, barriers made of polyvinyl chloride (PVC) plates were inserted into the depth of 0.5 m around each plot to prevent surface runoff and lateral movement of water between the plots and the surrounding soil. Water was applied to DW and WW plots using sprinkler systems consisting of nine sprayers, which were uniformly distributed at 1 m height. The water used for the sprinkler system was ground water from a nearby deep well (Yu et al. 2019, 2020).

Precipitation manipulation treatments were first carried out in 2013 and continued annually until 2017. To reduce throughfall in the DW plots, we used rainout shelters (2.5 m high at the apex) consisting of transparent flexible PVC sheets supported by a stainless steel framework (Fig. 1A,B). When unfolded from April to May, the sheets covered 60% of the plot area, which delayed the onset of the wet season by two months (Fig. 1A). In other months, the sheets were folded (Fig. 1B) allowing throughfall onto the plots. To delay the end of the wet season, each DW plot was irrigated once a week with equivalent amount in October and November (eight times in total; Fig. 1C). The total amount of water added to the DW plots was equivalent to 60% of the throughfall during April and May. Specifically, the amount of water added into the DW plots in the early dry season was 239 mm and 192 mm in 2015 and 2017, respectively (Fig. S1). Thus, the DW treatment reduced throughfall by *c*. 60% during eight weeks at the start of the wet season and supplemented throughfall by *c*. 60% during eight weeks at the start of the dry season, effectively delaying the wet season by two months.

For the WW treatment, 400 mm (*c.* 25% of mean annual precipitation) water was added in each plot in July and August. The irrigation was carried out once a week with 50 mm of water over a fourhour period (eight times in total).

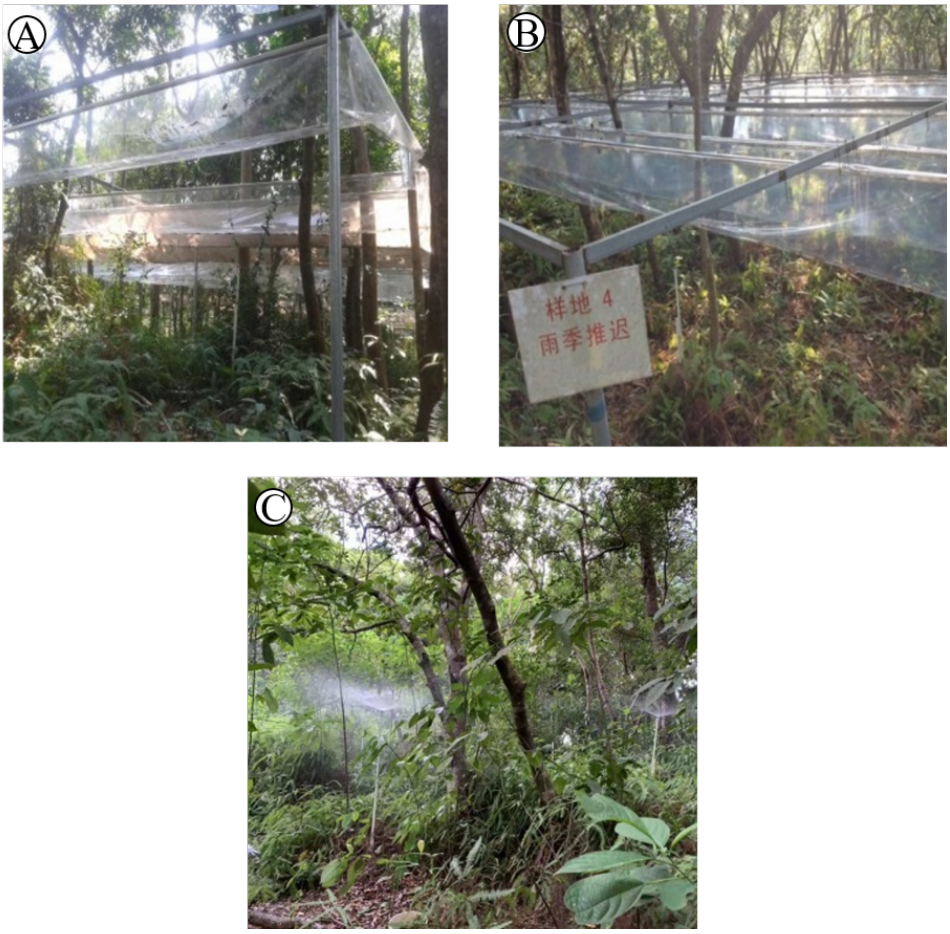

Figure 1 Precipitation manipulation plots in a seasonally dry tropical forest in South China, showing A) opened rainout shelters; B) folded rainout shelters; C) sprinkler irrigation system spraying water.

2.3 Soil sampling and soil and plant characteristics

Soil and plant characteristics were measured in January, April, August, and November of 2015 and 2017, representing the mid-dry season (MDS), early wet season (EWS; when througfall was reduced to delay the onset of the wet season in the DW plots), mid-wet season (MWS; when water was added in the WW plots) and early dry season (EDS; when water was added to delay the end of the wet season in the DW plots), respectively. At each timepoint, we randomly chose two sampling sites (*c.* 2-m × 2-m each) in each plot located at least 2 m from the edge of the plot to reduce trenching and edge effects. Within each sampling site, we took six soil cores (0-10 cm depth) using a drill sampler (5 cm internal diameter). The soils were returned to the laboratory, where live fine roots (diameter ≤ 2 mm) were picked out by hand, washed to remove the soil attached on surface, dried at 60 ℃ for 48 h and weighed to determine fine root biomass (FRB). After removing roots and other plant residues, the soil samples were immediately homogenized by sieving (2-mm mesh), which removed root fragments or stones. Gravimetric soil water content (SWC) was measured on ~20 g soil dried at 105 °C for 48 h. Dissolved organic C (DOC) and N (DON) were extracted in 0.5M K_2SO_4 solution and determined using a TOC analyzer (TOC-VCSH, Shimadzu GmbH). Extractable phosphorus (P_{extr}) was extracted in 0.5M NH_4F solution and measured using the molybdate blue method (Hou et al., 2014). Soil pH was determined on air-dried soils in a 1:2.5 (g:mL) mixture of soil to deionized water. Forest canopy leaf area index (LAI) was measured *in situ* before sunrise on days without rainfall using a Plant Canopy Analyzer (LAI 2000, LI-COR Biosciences, Lincoln, NE, USA). For initial calibration, 10 measurements were taken at an open field near our experimental site, and then at least 30 measurements were taken below canopy level at 2-m height in each plot.

2.4 Soil net N mineralization

We measured soil net N mineralization (net N_{min}) rates in January, April, August, and November in 2015 and 2017. In each sampling site, two soil cores (4.6 cm diameter × 15 cm height) were collected at the beginning of the month. One core from each pair was immediately returned to the laboratory, sieved to 2-mm, and processed for pre-incubation measurements of gravimetric soil water content and inorganic N. Nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) were extracted with 2M KCl solution within 48 h of soil sampling. The second core was incubated for one month *in situ* in a PVC pipe with a lid to prevent leaching by throughfall, and four holes (2 mm diameter) in the side walls for aeration. After the incubation, the soil cores were collected, returned to the laboratory, and SWC, NO_3^--N and NH_4^+-N were measured. The net N_{min} rate was calculated from the difference in pre- and post-incubation NO_3^--N and NH_4^+-N content. The mean N_{min} rates per plot were calculated from the two sampling sites and used for all data analyses ($n = 4$ per treatment).

2.5 Soil microbial biomass and community structure

We assessed microbial biomass and community structure by phospholipid fatty acid (PLFA) analysis following the procedure described by Bossio and Scow (1998). An 8-g aliquot of soil from each subplot was vacuum freeze-dried and stored at -20 ℃ for further analysis. The lipids in each freeze-dried subsample were extracted in a single-phase mixture of chloroform:methanol:phosphate buffer (1:2:0.8 by vol.; pH 7.4), and the extracts were analyzed with a gas chromatograph equipped with a flame-ionization detector (Agilent 6890, Agilent Technologies, Palo Alto, CA, USA). We used total microbial PLFAs to represent microbial biomass (Bardgett and McAlister, 1999) and grouped PLFAs to indicate soil microbial guilds based on fatty acid molecular structure. Gram positive bacteria were represented by PLFAs i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0, whereas Gram negative bacteria were represented by PLFAs 16:1ω7c, cy17:0, 18:1ω5c, 18:1ω7c and cy19:0 (Frostegard et al., 1993; Zelles, 1999). We further used PLFAs 10Me16:0, 10Me17:0 and 10Me18:0 to indicate actinomycetes (Cusack et al., 2011), PLFAs 18:1ω9 and 18:2ω6,9 to indicate fungi (Frostegard et al., 1993), and 16:1ω5c to represent arbuscular mycorrhizal fungi (Olsson et al., 1999).

2.6 Statistical analysis

Data was transformed where necessary to meet assumptions of normality. To assess the effects of precipitation manipulation treatment on soil net N_{min}, total soil microbial PLFAs, soil physicochemical attributes (SWC, pH, DOC, DON, NO_3^--N, NH_4^+-N and P_{extr}) and plant characteristics (FRB and LAI) over the experimental period, we used repeated measures ANOVA (RM ANOVA), with year and season as within-subject factors and treatment as between-subject factor. Due to the high seasonal variations (Table S1), we further examined the treatment effects for each season using RM ANOVA with year as a within-subject factor and treatment as between-subject factor (Table 1). Where the overall analyses were significant, we used Tukey's HSD post-hoc tests for pairwise comparisons among treatments. The RM ANOVAs were performed in SPSS 20.0 (IBM Corp, Armonk, NY, USA).

We assessed the effects of the precipitation manipulation treatments on soil microbial community structure using multivariate analyses based on individual PLFA mass contents in the vegan package (Oksanen et al., 2019) in R 4.0.2 (Team, 2020). The PLFA mass data were scaled and centered before visualizing the separation of communities among treatments using principal component analysis (PCA; *rda* function). We tested for differences in microbial communities among treatments using permutational multivariate analysis of variance (PERMANOVA, *adonis* function). For seasons in which microbial community composition differed among treatments, we then explored the influence of soil and plant properties on soil microbial community composition using redundancy analysis (RDA) with backward step-wise selection of *a priori* explanatory variables (SWC, pH, DOC, DON, DOC:DON ratio,

NO_3^--N, NH_4^+-N, P_{extr}, fine root biomass and LAI).

To determine relationships between plant or soil variables and soil net N_{min} or microbial biomass, we used linear models with all-subset selection of *a priori* explanatory variables (SWC, pH, DOC, DON, DOC:DON ratio, NO_3^--NNO, NH_4^+-N, P_{extr}, fine root biomass and LAI) in the leap package (Lumley and Miller, 2020) in R. Given the importance of microbial biomass and community composition for net N_{min}, the *a priori* explanatory variables for net N_{min} also included microbial biomass and PC1 (the first principal component from the PCA).

Finally, we used the results of the linear models for net N_{min} to explore potential underlying mechanisms contributing to changes in net N_{min} rates in the early dry season, using structural equation models (SEM) in AMOS 17.0 (Amos Development, Spring House, USA). The initial SEM was based on *a priori* assumptions about the importance of soil microbial community structure, soil physicochemical attributes and plant characteristics for net N_{min} rates. To determine the relative importance of soil microbes in regulating N_{min} under changing precipitation regimes, microbial biomass and community structure (represented by PC1) were linked to net N_{min} in the *a priori* SEM by default. The explanatory variables from the final linear model for N_{min} were then included as factors directly influencing net N_{min}. However, as we were principally interested in understanding the impact of changing precipitation regime, we excluded variables that were not related to SWC or affected by the experimental treatments (i.e. where significance was $p > 0.1$). Similarly, we used the results of the linear regressions to include links between explanatory variables and microbial biomass or community structure. We then tested alternative SEM models using stepwise removal of non-significant paths. The best-fit model was determined using maximum likelihood χ^2 tests, the root-mean-square error of approximation (RMSEA) index and the goodness-of-fit index (CFI) (Grace et al., 2010).

3 Results

3.1 Soil and plant characteristics

Across our eight sampling timepoints, SWC was 12.9-26.5% higher in the DW plots than in the controls but the increase was only significant in the early dry season and mid-wet season (RM ANOVA: $p < 0.05$; Table 1, Fig. 2A). Interestingly, SWC in the DW plots did not decline with throughfall reduction in the early wet season. By contrast, SWC only increased in the WW plots during the mid-wet season, when the irrigation treatment was active ($p = 0.07$; Table 1; Fig. 2A). Soil water content was 17.2% higher in the WW treatment compared to the controls in the August in 2015 ($p = 0.17$) and 26.0% higher in 2017 ($p < 0.01$; Fig. 2B). Hence, a delayed wet season increased SWC to a greater extent than a wetter wet season.

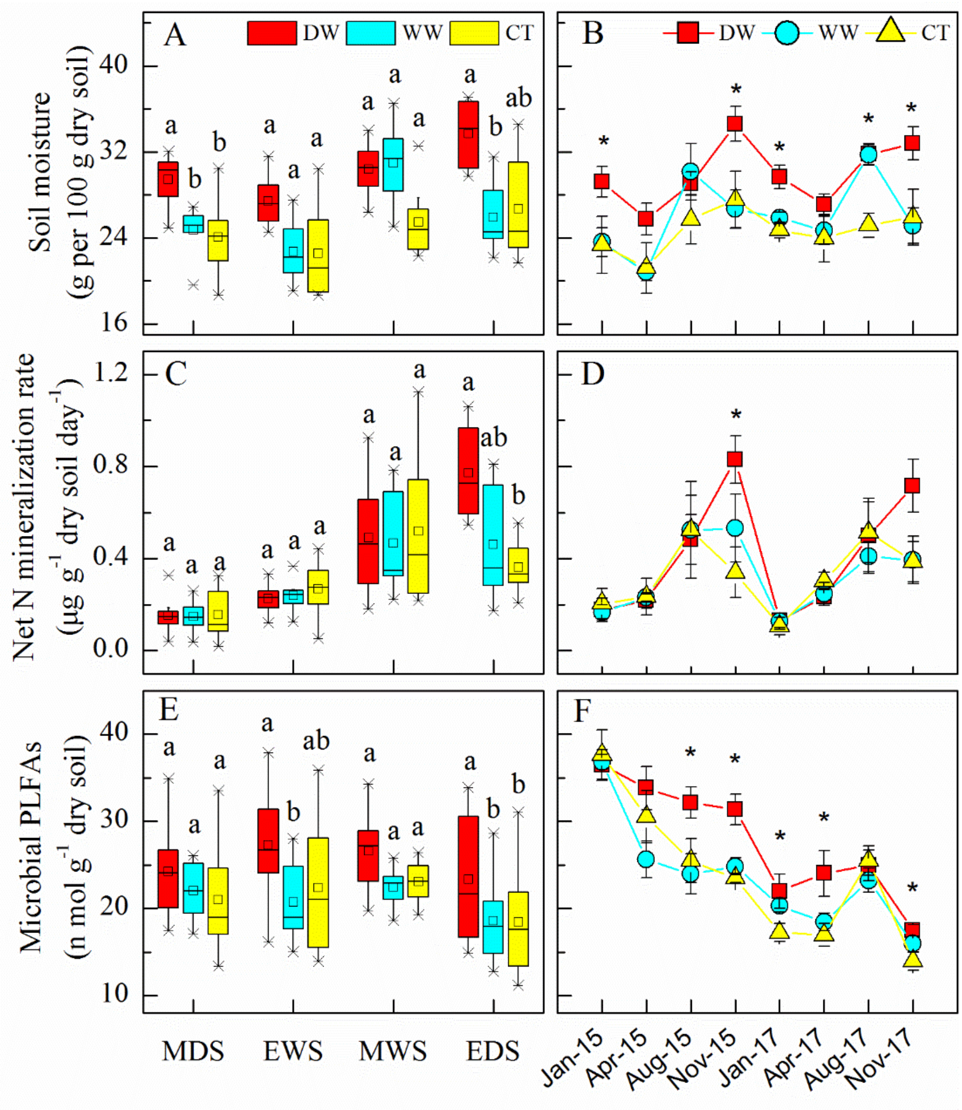

Figure 2 The influence of precipitation manipulation treatments on (A, B) gravimetric soil water content, (C, D) soil net N_{min} rate, and (E, F) microbial biomass (total PLFA content) during seasonal periods (A, C, E) and at eight sampling timepoints (B, D, F) and in a seasonally dry tropical forest; treatments are delayed wet season (DW) or a wetter wet season (WW) and controls (CT). Seasonal periods are mid-dry season (MDS), early wet season (EWS), mid-wet season (MWS) and early dry season (EDS). Boxplots with means (squares) are shown for $n = 8$ (A, C, E) per treatment, and means (symbols) ± standard errors are shown for $n = 4$ (B, D, F). Asterisks and different letters represent statistically significant differences at $\alpha = 0.05$.

Table 1 Results of repeated measures ANOVA examining the effects of year and precipitation change on soil net N mineralization, soil microbe and soil and plant characteristics in each seasonal period

		SWC	N_{min}	Total PLFAs	pH	DOC	DON	DOC: DON ratio	NO_3^--N	NH_4^+-N	P_{extr}	LAI	FRB
MDS	Year	0.25	0.13	*<0.01*	*<0.01*	*<0.01*	*0.02*	0.20	0.43	0.43	*<0.01*	0.57	0.43
	Treatment	*0.01*	0.98	0.42	0.13	0.07	0.17	0.28	0.91	0.41	0.99	*<0.01*	*0.03*
	Year×Treatment	0.81	0.78	0.42	0.16	*0.02*	0.66	0.69	0.99	0.99	0.29	0.87	0.74
EWS	Year	*0.02*	0.16	*<0.01*	<0.01	*<0.01*	*0.02*	*<0.01*	0.46	*0.01*	*0.01*	1.00	0.70
	Treatment	0.08	0.78	*0.02*	0.21	0.10	*0.01*	0.37	0.24	0.03	*0.01*	*0.02*	*0.01*
	Year×Treatment	0.07	0.57	0.44	0.38	0.35	0.36	0.17	0.12	0.27	0.89	0.99	0.95
MWS	Year	0.19	0.75	*0.01*	*0.01*	*<0.01*	*<0.01*	0.96	0.48	*0.01*	*0.01*	0.79	0.53
	Treatment	0.05	0.94	0.09	0.18	0.42	0.53	0.08	0.48	*0.02*	*0.01*	0.07	0.23
	Year×Treatment	0.33	0.88	*<0.01*	0.18	0.33	0.38	0.28	0.44	0.94	0.52	0.99	0.52
EDS	Year	0.05	0.25	*<0.01*	*<0.01*	*<0.01*	*<0.01*	0.93	*0.01*	*<0.01*	*<0.01*	0.64	*<0.01*
	Treatments	*0.04*	*0.03*	*0.02*	*0.04*	0.05	0.16	*0.04*	0.86	0.72	0.26	*0.03*	0.17
	Year×Treatment	0.99	0.39	0.22	0.68	0.09	0.87	0.78	0.25	0.32	0.63	0.40	0.66

Notes: SWC is soil gravimetric water content; N_{min} is soil net N mineralization rate; Total PLFAs is total soil microbial phospholipid fatty acid; pH is soil pH; DOC is dissolved organic C; DON is dissolved organic N; DOC:DON ratio is the ratio of DOC to DON; NO_3^--N is nitrate-N; NH_4^+-N is ammonium-N; P_{extr} is extractable phosphorus; LAI is forest canopy leaf area index and FRB is fine root biomass. Seasonal periods are mid-dry season (MDS), early wet season (EWS), mid-wet season (MWS) and early dry season (EDS). Significant effects are highlighted in italic.

Over the two-year experimental period, soil pH was significantly higher in the WW than in the DW plots (*p* = 0.03; Table S1; Fig. 3A). The effects of the treatments on DOC (*p* = 0.02), DON (*p* = 0.03), LAI (*p* < 0.01) and P_{extr} (*p* < 0.01) depended upon the seasonal period in which samples were collected (Table S1). Dissolved organic carbon (DOC) tended to be lower in WW plots than in the other treatments in the mid-wet season (*p* < 0.12) and early dry season (*p* < 0.07), while DOC tended to be higher in DW plots in the early dry season (*p* < 0.16; Fig. 3B). Similarly, DON tended to be lower in WW plots than the other treatments in the mid-wet season (*p* < 0.13; Fig. 3C), while DON was significantly higher in DW plots in the early wet season (*p* < 0.04). The DOC:DON ratio, NO_3^--N and NH_4^+-N did not differ significantly among treatments (Fig. 3D,E,F). However, soil P_{extr} was significantly higher in the DW plots compared to the other treatments in the mid-wet season (*p* < 0.01) and mid-dry season (*p* < 0.03; Fig. 3G) , and soil P_{extr} in the WW plots was lower than in the DW plots in the early wet season (*p* = 0.02). Interestingly, LAI was highest in the DW plots in the mid-dry season, whereas the highest values of LAI in the other treatments occurred in the mid-wet season, indicating a substantial but lagged response to irrigation in the early dry season in the DW plots (*p* = 0.02; Fig. 3H). Consequently, LAI was significantly higher in the DW plots than in the other treatments in the mid-dry season (*p* < 0.01) and slightly higher in the early wet season (*p* = 0.06), but significantly lower than WW plots in early dry season (*p* = 0.03). In addition, precipitation change significantly affected fine root biomass over the two-year experimental period (RM ANOVA: *p* = 0.03; Table S1). The results showed that fine root biomass was lower in the WW plots than in the controls (*p* = 0.02; Fig. 3I).

3.2 Soil net N mineralization

The effect of DW in net N_{min} was dependent on sampling season (*p* = 0.05; Table S1). Net N_{min} rates were significantly accelerated by the DW treatment in the early dry season (*p* = 0.03) but were not affected by the WW treatment in any sampling point (Table 1; Fig. 2C,D). The net N_{min} rate in the DW plots (0.83 ± 0.10 μg g^{-1} dry soil d^{-1}) was more than double the rate in the CT plots in November 2015 (0.34 ± 0.11 μg g^{-1} dry soil d^{-1}; *p* = 0.02) and twice as high as in the CT plots in November 2017 (0.72 ± 0.12 vs. 0.39 ± 0.09 μg g^{-1} dry soil d^{-1} in DW and CT plots, respectively (*p* = 0.12; Fig. 2D).

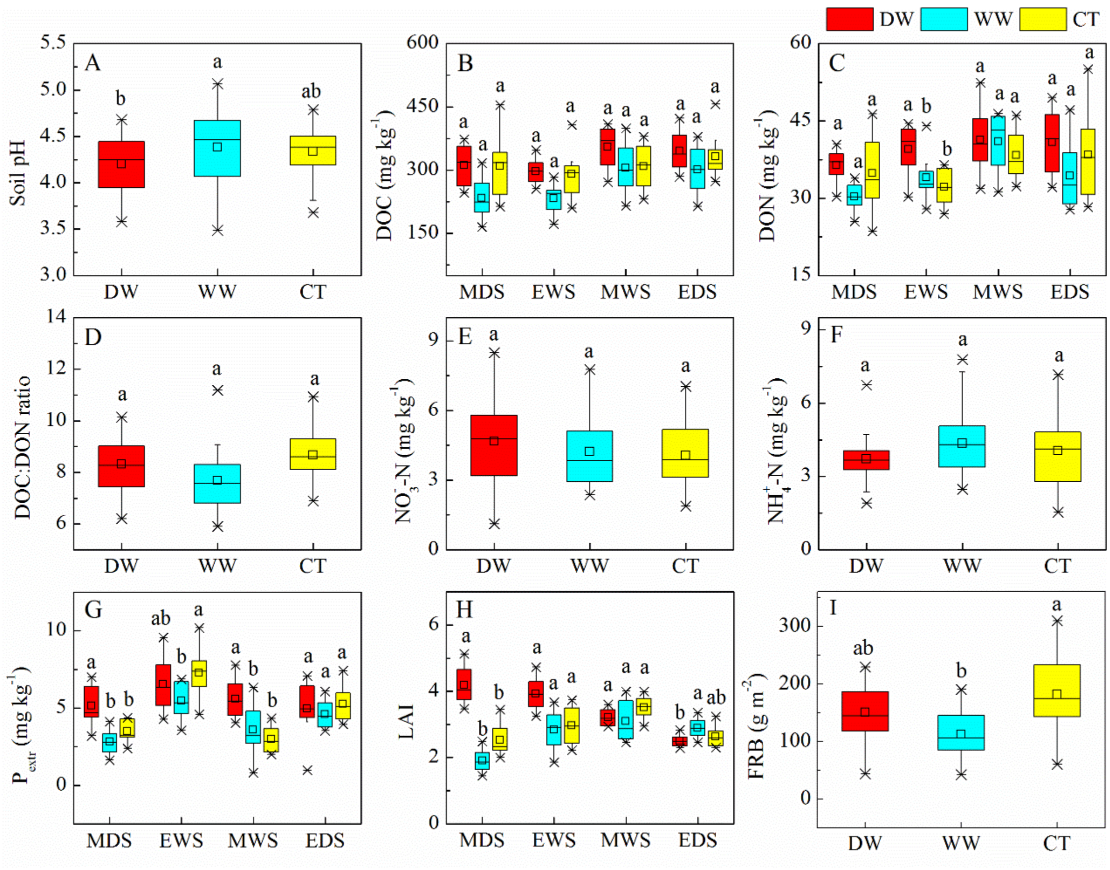

Figure 3 The influence of precipitation manipulation treatments on A) soil pH, B) dissolved organic C (DOC), C) dissolved organic N (DON), D) DOC:DON ratio, E) nitrate-N (NO_3^--N), F) ammonium-N (NH_4^+-N), G) extractable phosphorus (P_{extr}), H) forest canopy leaf area index (LAI) and I) fine root biomass (FRB). Treatment comparisons across both sampling years are shown, and seasonal data are shown where repeated measures ANOVA revealed a significant treatment × season interaction (B,C,G,H). Treatments are delayed wet season (DW) or a wetter wet season (WW) and controls (CT). Seasonal periods are mid-dry season (MDS), early wet season (EWS), mid-wet season (MWS) and early dry season (EDS). Boxplots with means (squares) are shown for $n = 8$ (B, C, G, H) or $n = 32$ (A, D, E, F, I) samples (4 plots per treatment and 2 or 4 time-points). Different letters represent statistically significant differences at $\alpha = 0.05$. Data for soil pH, DOC, LAI and FRB in 2015 were previously published (Yu et al., 2020).

3.3 Soil microbial communities

Over the two-year experiment, precipitation change treatments affected microbial biomass (total PLFAs; RM ANOVA: $p = 0.01$; Table S1). Soil microbial biomass was significantly higher in the DW plots than controls in the mid-wet season in 2015 and the early wet season and early dry season in 2017 ($p < 0.05$; Fig. 2F). Across both years, microbial biomass increased significantly in DW plots compared to controls in the early dry season ($p = 0.02$) and there was a non-significant trend towards higher microbial biomass in the early wet season (RM ANOVA: $p = 0.07$) and the mid-wet season (RM ANOVA: $p = 0.10$; Table 1; Fig. 2E). By contrast, the WW treatment did not affect microbial biomass at any sampling point or during any of the seasonal periods (Table 1; Fig. 2E,F).

In contrast to the response of microbial biomass, soil microbial community composition was mainly affected by precipitation changes in the early dry season. The PCA ordination plot showed a clear separation of the soil microbial communities in DW plots and controls in November of 2015 and 2017 (Fig. 4), and the PERMANOVA revealed significant differences in soil microbial communities between DW and WW plots in early wet season 2015 ($r^2 = 0.52$, $p = 0.02$) and between DW and CT plots in November of both years (2015: $r^2 = 0.63$, $p = 0.03$ and 2017: $r^2 = 0.50$, $p = 0.02$; Table S4). However, no specific microbial guild contributed disproportionately to the change in community structure, as neither the relative abundance nor the total amount of PLFAs for any guild changed in the DW plots during the early dry season (Fig. S2,3).

3.4 Relationships between microbial communities or net N_{min} rates and plant or soil properties

The redundancy analysis assessing relationships between microbial communities and plant or soil variables in the early dry season included soil pH ($r = 0.063$, $p = 0.09$), FRB ($r = -0.003$, $p < 0.01$), NO_3^--N ($r = -0.005$, $p = 0.06$) and the DOC:DON ratio ($r = -0.013$, $p = 0.01$), which together explained 26% of the variance in soil microbial community composition ($p < 0.01$, Fig. 5).

The linear models revealed relationships between microbial biomass or net N_{min} rates and several plant or soil variables during the early dry season. The best model for microbial biomass included LAI ($r = 2.73 \pm 1.347$, $p = 0.06$), SWC ($r = 2.73 \pm 1.347$, $p < 0.01$), FRB ($r = 0.05 \pm 0.02$, $p < 0.01$), DON ($r = -0.53 \pm 0.12$, $p < 0.01$), NH_4^+-N ($r = -1.00 \pm 0.93$, $p = 0.30$) and P_{extr} ($r = -1.92 \pm 0.72$, $p = 0.02$), which together explained 76% of the variance ($p < 0.01$; Table S5). The best model for soil net N_{min} explained 39% of the variance ($p = 0.01$; Table S5). Soil net N_{min} significantly increased with increasing SWC ($r = 0.31 \pm 0.01$, $p < 0.01$), but declined with increasing NH_4^+-N ($r = -0.13 \pm 0.03$, $p = 0.04$) and DOC:DON ratio ($r = -0.13 \pm 0.05$, $p = 0.02$). The model also included PLFAs ($r = 0.02 \pm 0.01$, $p = 0.06$) and LAI ($r = -0.16 \pm 0.01$, $p = 0.09$).

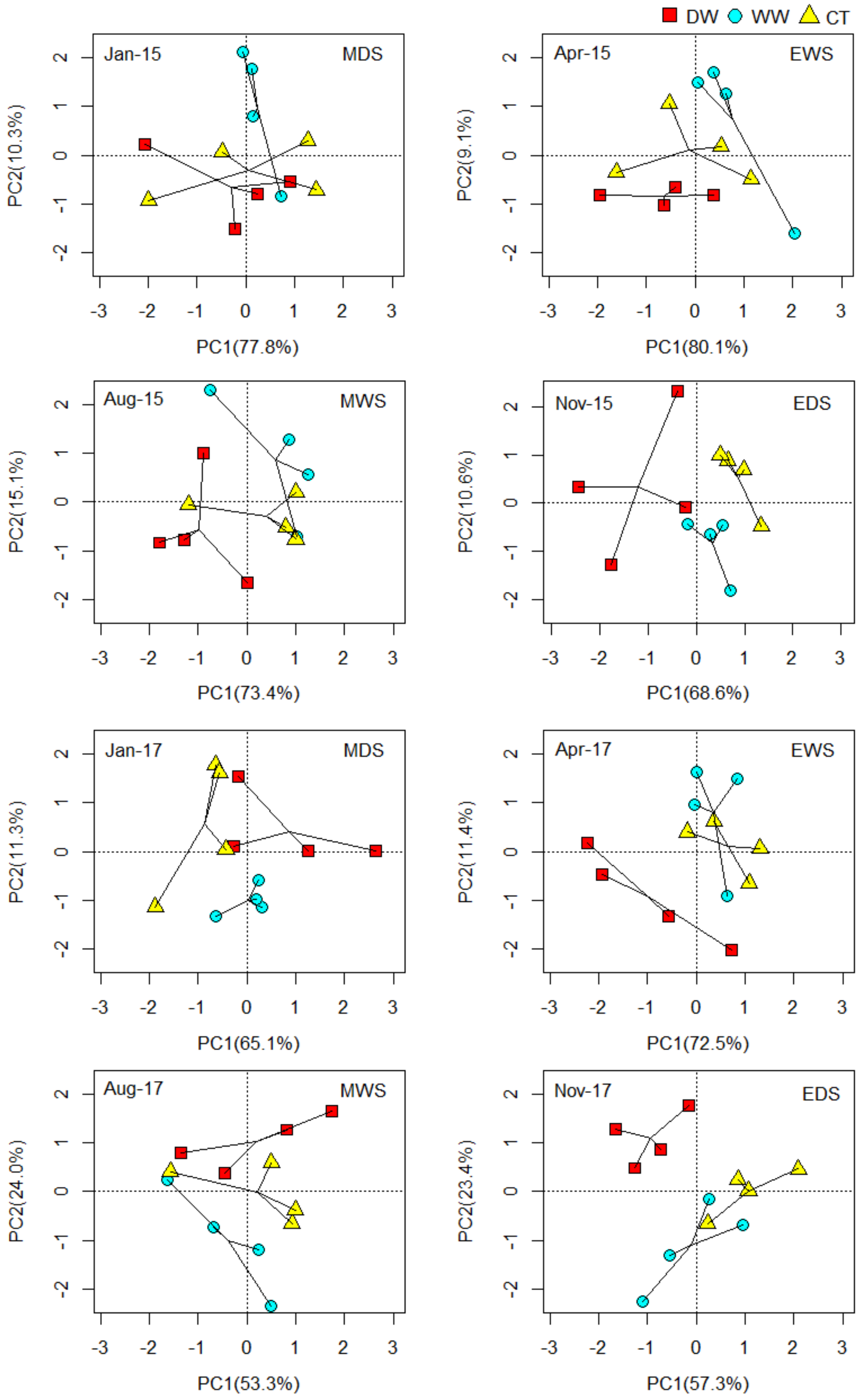

Figure 4 Ordination plots from principal component analysis showing changes in soil microbial communities at each sampling time in treatments simulating a delayed wet season (DW) or a wetter wet season (WW) and controls (CT). Seasonal periods are mid-dry season (MDS), early wet season (EWS), mid-wet season (MWS) and early dry season (EDS).

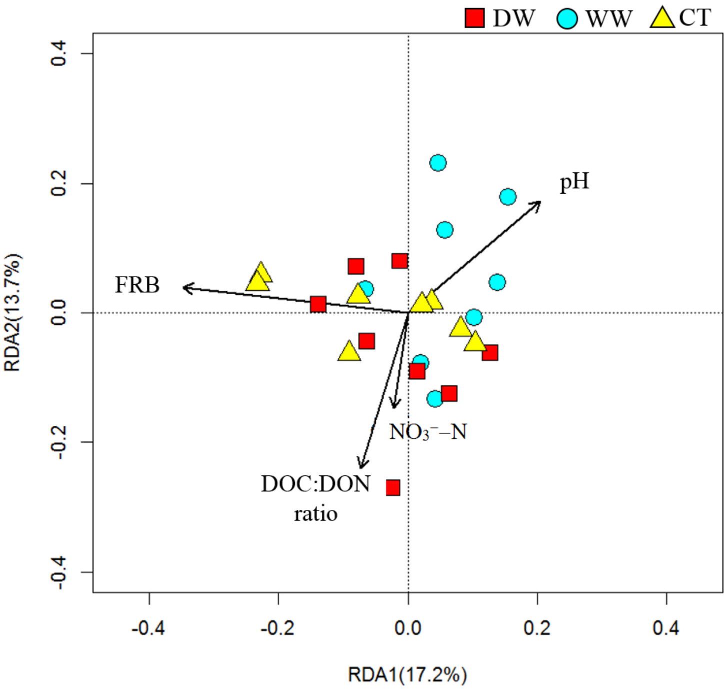

Figure 5 Redundancy analysis (RDA) ordination plot of soil microbial communities in the early dry season in treatments simulating a delayed wet season (DW) or a wetter wet season (WW) and controls (CT), based on (RDA), showing the influence of plant or soil properties on community composition. Arrows indicate vectors of the variables included in the best RDA model, where pH is soil pH; DOC:DON ratio is the ratio of dissolved organic C to dissolved organic N; NO_3^--N is nitrate-N and FRB is fine root biomass.

3.5 Structural equation model

Our SEM explained 45% of the variance in soil net N_{min} rate in the early dry season (Final model fit: χ^2 = 10.5, p = 0.65, *d.f.* = 13, CFI = 0.74, RMSEA < 0.01; Fig. 6). Direct paths in the SEM showed that net N_{min} rates increased with microbial biomass (path = 0.30, p = 0.08), but declined with DOC:DON ratio (path = -0.42, p = 0.02), LAI (path = -0.39, p = 0.03) and PC1 representing microbial community structure, albeit not significantly (path = -0.25, p = 0.16). Interestingly, although the precipitation manipulation treatments were associated with changes in SWC (path = -0.57, p < 0.01), differences in net N_{min} rates were not directly associated with SWC. Instead, the indirect relationship between SWC and N_{min} rates was mediated by several other variables: higher SWC was associated with increasing DON (path = 0.37, p = 0.05) and microbial biomass (path = 0.85, p < 0.01) but declining LAI (path = -0.73 p < 0.01), all of which were direct or directly related to net N_{min} rates. In addition, the relationship between net N_{min} and the DOC:DON ratio was indirectly associated with SWC via LAI (path = -0.48, p =

0.01).

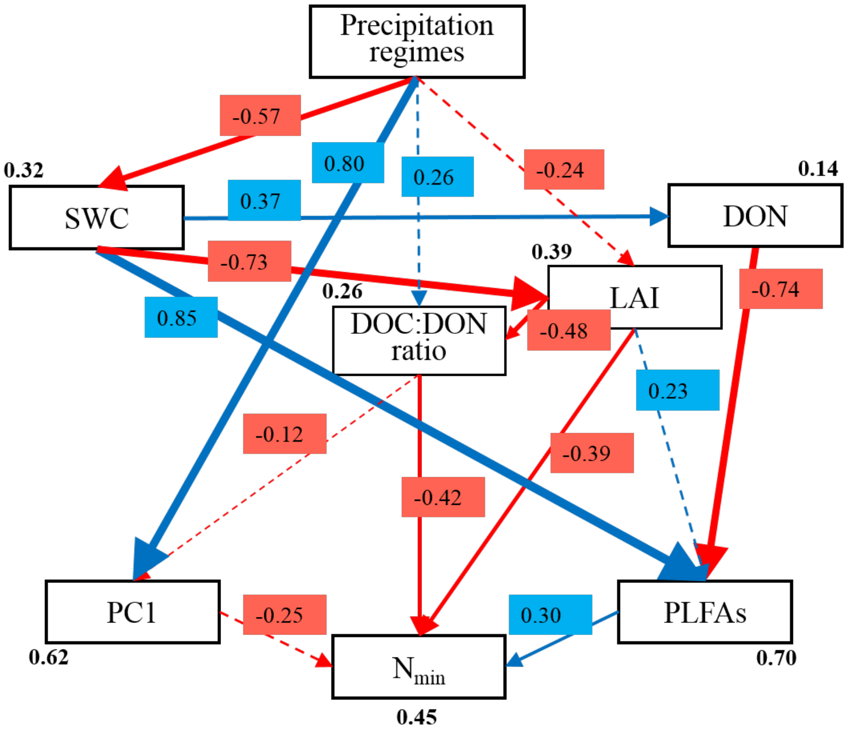

Figure 6 Final structural equation model (SEM) showing how changing precipitation regimes affect soil net N mineralization in the early dry season. Numbers next to arrows indicate the standardized parameter for each path. Squared multiple correlations for each predictor variable are shown in bold next to boxes. Solid arrows indicate significant paths at $p < 0.05$, and dashed lines are non-significant paths ($p \geq 0.05$) retained in the best fit model. SWC is soil water content; DON is dissolved organic N; DOC:DON ratio is the ratio of DOC to DON; NO_3^--N is nitrate-N; LAI is forest canopy leaf area index; PC1 is the first principle component representing microbial community composition; PLFAs is total phospholipid fatty acids representing soil microbial biomass; N_{min} is soil net N mineralization.

4 Discussion

Whereas most previous studies investigating the effects of precipitation change on net N_{min} have investigated the effects of increased or reduced annual precipitation, our experiment focused on changes in seasonal precipitation regime, which may be particularly important in seasonally dry tropical forests (Allen et al. 2017). Our findings demonstrated that a delayed wet season had a much greater impact on soil net N_{min} and soil microbial communities than increased precipitation during the wet season. In addition, the impact of altered precipitation regime on net N_{min} rates was mediated by soil and plant properties, and only indirectly associated with SWC. Hence, our study suggests that shifts in the timing of rainfall are likely to have greater consequences for the functioning of seasonally dry tropical forests than a wetter wet season.

4.1 The effects of a wetter wet season

In tropical forests, soil net N_{min} usually declines with increasing mean annual precipitation (Santiago et al., 2005; Alvarez-Clare and Mack, 2011) and soil water availability plays an important role in regulating net N_{min} under precipitation changes (Jamieson et al., 1998; Emmett et al., 2004; Zhu et al., 2017). However, most information about the influence of precipitation change on net N_{min} rates comes from natural rainfall gradients, and there are few experimental studies investigating how increased wet season precipitation will influence net N_{min}.

The present study suggested that increased total annual precipitation by *c.* 25% in the mid-wet season could increase soil moisture. However, our findings were in contrast to the general patterns across rainfall gradients because increased wet season precipitation had no effect on net N_{min} rates. Similarly, Ploughe and Dukes (2019) found that a wetter summer did not affect soil net N_{min} in a temperate forest in north America, suggesting that the effects of precipitation change on net N_{min} are seasonally dependent (Gutknecht et al., 2012; Sorensen et al., 2013). Instead, our study revealed that the relationship between SWC and net N_{min} rates was mediated by plant and soil properties, as well as soil microbial community composition and hence that both above- and belowground responses to altered precipitation patterns could have wide-reaching consequences for nutrient dynamics in seasonally dry tropical forests (Lodge et al., 1994; Schimel and Bennett, 2004). N mineralization in the soil is regulated by soil microbial communities (Fierer and Schimel, 2002; Bengtsson et al., 2003; Smithwick et al., 2005; Ribbons et al., 2016; Li et al., 2019). Hence, the lack of changes in soil microbial biomass or community composition in the WW treatment in our study indicates that the soil system in our study forest was resistant to increased soil moisture during the wet season. To our knowledge, there is no other direct evidence indicating that soil microbe in seasonally dry tropical forests is resistant to high soil moisture in the wet season. However, there are studies in other regions suggesting that increased precipitation does not change soil microbial biomass or community structure (Cruz-Martinez et al., 2009; Bell et al., 2014). Moreover, it is found that previous precipitation condition could shape microbial resilience and resistance to precipitation changes (Hawkes and Keitt, 2015; Averill et al., 2016). In the study region, it usually rains heavily in the mid-wet season and soil microbes in these forests may have a strong capacity to adapt to high soil moisture levels and even short-term waterlogging, which would explain why our WW treatment had little effect. Overall, our findings suggest that the total amount of rainfall during wet season is less important than the timing of the wet and dry seasons in regulating net N_{min} in seasonally dry tropical forests.

4.2 The effects of a late wet season

To our knowledge, this is the first study examining the effects of a delayed wet season on soil net N_{min} in a seasonally dry tropical forest. Notably, our findings suggest that greater SWC due to a delayed wet season substantially affected soil microbial communities and soil and plant characteristics during the early dry season, which in turn resulted in a substantial increase in net N_{min}. Soil processes in seasonally dry tropical forests are often strongly limited by water availability during the dry season in seasonally dry tropical forests (Jaramillo et al., 2011; Allen et al., 2017). For example, dry-season irrigation accelerated litter decomposition in Amazonia (Vasconcelos et al. (2007) and Panama (Wieder and Wright, 1995), even though these forests experience a weaker dry season than the forest at our study site. Hence, increased soil moisture due to irrigation during the early dry season likely alleviated water limitation to soil microbes in our study, resulting in higher rates of net N_{min} (Austin et al., 2004; Anaya et al., 2007). However, it is noteworthy that net N_{min} rates did not differ from the controls or WW plots at other sampling times, suggesting that total annual net N_{min} might be higher in the DW plots, and therefore that a delayed wet season could accelerate N cycling in this seasonally dry tropical forest. We expected lower SWC and net N_{min} in the DW plots in April, when throughfall was being excluded. However, the increase in soil moisture in the DW plots was still apparent through the mid-dry and early wet season, suggesting that the shift in the end of the wet season, rather than its late onset, influenced net N_{min} and microbial communities. It is also possible that the installation of belowground barriers around the plots helped maintain soil moisture levels by reducing uptake by surrounding trees, a phenomenon observed in trenching experiments (Sayer and Tanner, 2010). However, we saw no similar effect on soil moisture or net N_{min} as a result of the same barriers in the WW plots and thus, the installation of belowground barriers does not fully explain the sustained higher SWC in the DW plots.

The increase in microbial biomass with a delayed wet season was also sustained during most of the year in 2017, which highlights the vital role of soil moisture in regulating soil microbes (Wagener and Schimel, 1998; Bardgett et al., 1999; Fierer et al., 2003; Brockett et al., 2012; Manzoni et al., 2012). Overall, microbial biomass in the DW plots largely mirrored the temporal patterns in SWC, but as water was unlikely to be limiting to microbial communities during the wet season (Allen et al., 2017; Zhao et al., 2017), we only observed a significant positive relationship between SWC and microbial biomass in the dry season (data not shown). Interestingly, microbial biomass in the DW plots was higher than the other treatments even in August 2015 and April 2017, when soil moisture did not differ among treatments. Hence, a delayed wet season appears to improve soil moisture through much of the year, supporting a larger microbial biomass.

Across both years, we found little evidence for a close relationship between soil microbial biomass and net N_{min} rate, although studies in other ecosystems describe an increase in net N_{min} with greater soil microbial biomass (Bengtsson and Bergwall, 2000; Remy et al., 2018; Li et al., 2019). However, increased soil microbial biomass could also entail a stronger demand for N, leading to a slower release of N (Baer et al., 2003; Schimel and Bennett, 2004). In addition, N leakages during the processes of nitrification and denitrification would increase with increased microbial activity thus diminishing the N retained in soils (Chapin et al., 2011) and decoupling the relationship between soil microbial biomass and net N_{min}. By contrast, we found a strong relationship between soil microbial community structure and net N_{min} during the early dry season, which has also been observed in studies in other ecosystems (Balser and Firestone, 2005; Ribbons et al., 2016; Remy et al., 2018). However, it is unclear which microbial guilds were responsible for the increase in net N_{min} in the DW plots because the biomass of all microbial guilds increased under the DW treatment. Hence, our study suggests that microbial community structure plays an important role in regulating the effect of shifting precipitation patterns on net N_{min} rates and detailed studies using molecular techniques could improve our mechanistic understanding of N cycling in seasonally dry tropical forests under future climate change.

4.3 Underlying mechanisms regulating changing precipitation effects on soil net N mineralization

A delayed wet season increased soil net N_{min} in the early dry season, and our SEM suggested that the soil microbial community, resource stoichiometry and forest canopy LAI jointly affected soil net N_{min}. The DOC:DON ratio had the biggest direct effect on N_{min}, illustrating the importance of resource stoichiometry (Sterner and Elser, 2002). The C:N ratio of substrates can determine microbial N mineralization outcomes (Manzoni et al., 2008) and dissolved organic matter is readily available as a substrate for microbial use(Chapin et al., 2011). Consequently, the C:N ratio of dissolved organic matter (DOC:DON) will play a key role in regulating soil net N_{min} rates. Our results, indicating that N_{min} decreased with increasing DOC:DON, suggest that microbial N transformation becomes more limited by N as the C:N ratio increases, which is consistent with previous studies (Mooshammer et al., 2012; Heuck and Spohn, 2016).

Forest canopy influences the amount and composition of aboveground litter fall, which largely determines the amount of nutrients to be mineralized and the resulting nutrient availability (Prescott, 2002). The LAI of the forest canopy is usually considered an indicator of forest productivity (Alvarez-Clare et al., 2013; Lim et al., 2015). However, the LAI at the start of the dry season also indicates how many leaves are left on trees at the early dry season, as LAI declines from the mid-wet season to the early dry season. Consequently, higher LAI in the early dry season would mean less leaf litter available to microbes, and lower inputs of C and N for microbial decomposers explains why the DOC:DON ratio and N_{min} rates declined with increasing LAI in our models. Nonetheless, in our previous work at the same site, we observed higher decomposition rates in the DW plots in the early dry season (Yu et al., 2020). Hence, the seasonal shift in precipitation boosted net N_{min} rates by maintaining SWC and litter decomposition at the start of the dry season.

Our SEM demonstrated that N_{min} rates were related to soil microbial biomass and community structure, highlighting the important role for soil microbes in regulating soil net N_{min} rates (Bengtsson and Bergwall, 2000; Remy et al., 2018), and the indirect effect of precipitation change on N_{min} rates via the relationship between microbial communities and SWC. Our SEM also showed that the links between water availability, microbial communities and N dynamics likely underpin the increase in soil net N_{min} in the DW plots during the early dry season, because most factors directly influencing N_{min} were related to SWC, which was, in turn, strongly influenced by the altered precipitation regimes. Hence, delaying the end of the wet season can substantially affect processes above- and belowground (Jaramillo et al., 2011; Allen et al., 2017) with knock-on effects for N cycling.

5 Conclusions

We demonstrate that a shift in the timing of the wet season has a greater impact on net N_{min} rates in seasonally dry tropical forests than changes in the amount of precipitation during the wet season. A two-month delay in the onset of the wet season resulted in higher SWC and microbial biomass during much of the year, as well as higher overall net N_{min}, whereas increased wet season precipitation had little effect. Our findings are important because we based our experimental treatments on climate model projections indicating an extended wet season in the study region in future. Collectively, the results from this unique experiment indicate that shifts in seasonality are altering elemental cycling in seasonally dry tropical forests. Future work should assess whether altered precipitation patterns create asynchrony between plant nutrient demand and microbial activity, which would affect overall ecosystem functioning.

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