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Microbial processing of plant remains is co-limited by multiple nutrients in global grasslands

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Microbial processing of plant remains is co-limited by multiple nutrients in global grasslands

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Abstract

Microbial processing of unprotected organic matter inputs is key for soil formation, long-term ecosystem carbon and nutrient sequestration, and a sustainable agriculture. We investigated the effects of adding multiple nutrients on decomposition and stabilization of standard plant materials buried in twenty-one grasslands from four continents that were fertilized with nitrogen, phosphorus, and potassium plus nine essential macro- and micronutrients. Addition of multiple nutrients weakly but consistently increased decomposition and biochemical stabilization of unprotected plant remains, mainly through changes in microbial ecoenzymatic activity. Higher precipitation and lower temperature of the wettest quarter were main drivers of higher decomposition rates, while lower temperatures of the wettest quarter enhanced the biochemical stabilization of plant remains. The enhancing effects of nutrients were greatest at sites with lower temperatures and higher rainfall, indicating a very limited potential of fertilized grassland soils to increase our ability to offset carbon emissions in a warmer and drier future.

Keywords: Carbon cycling and sequestration; Eutrophication; Fertilization; Microbial activity; NutNet; Nutrient (co-)limitation

Running head: Soil processing of plant matter in grasslands

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INTRODUCTION

Many ecosystems worldwide are receiving greater inputs of readily available nutrients due to increasing contributions from various anthropogenic sources (Sala *et al.*, 2000; Fowler *et al.*, 2013). For example, many grasslands are fertilized with nitrogen (N), phosphorus (P), potassium (K) and other essential macro- and micronutrients to improve pasture yield and nutritional quality (Conant *et al.*, 2001). Additionally, the non-intentional atmospheric and aeolian deposition of biologically-limiting nutrients is a common source of eutrophication in these ecosystems (Gruber & Galloway, 2008; Fowler *et al.*, 2013). Considered as a whole, natural, seminatural and anthropogenic grasslands cover a large proportion (~40%) of the global land surface, serve as a source of forage and food production, and sequester approximately 20-30% of all terrestrial C, most of it in the soil (Scurlock & Hall, 1998; Conant *et al.*, 2001; O'Mara, 2012). The rate of decomposition and biochemical stabilization of superficial and buried unprotected plant remains is a lynchpin for soil fertility and ecosystem-level carbon (C) fluxes in grassland ecosystems (Cadisch & Giller, 1997; Bradford *et al.*, 2016), hence for their sustainability. Thus, understanding how the simultaneous increase in multiple essential nutrients drives microbial processing of plant remains, and the modulating role of local climatic conditions in this process, is a crucial gap in our knowledge for predicting how both unmanaged and managed grasslands will function under ongoing and future global environmental change scenarios.

Break-down of physically unprotected plant organic matter inputs by detritivores and further decomposition by microbes is central to nutrient cycling and is the first step in the formation of soil organic matter (Cadisch & Giller, 1997). Decomposition of plant materials typically occurs in two phases (Cadisch & Giller, 1997). Initial decomposition rates are relatively

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high due to the breakdown of labile compounds, a process typically quantified by the exponential decomposition rate constant *k* (Cadisch & Giller, 1997). Later in the process, decomposition rates generally slow, stabilizing at a limit value (Berg *et al.*, 2003), as labile compounds are lost or transformed to recalcitrant compounds that accumulate together with microbial necromass (Bradford *et al.*, 2016). Also, soil microbial communities play an important role in these processes as they release extracellular enzymes that break down different types of plant materials (Philippot *et al.*, 2013; Leff *et al.*, 2015; Prober *et al.*, 2015). However, it is unknown how the release of soil microbial enzymes related to C, N and P cycles affects the rate at which different types of plant remains that vary in labile and recalcitrant fractions decompose (Wickings *et al.*, 2012). Moreover, the addition of many essential nutrients, including N, P, K, sodium (Na) and manganese (Mn), can accelerate initial decomposition rates (Hobbie & Vitousek, 2000; Knorr *et al.*, 2005; Kaspari *et al.*, 2008a, 2009; Keiluweit *et al.*, 2015; Ochoa-Hueso *et al.*, 2019a) and also decrease mass loss in later phases of decomposition (Berg, 2014), but global-scale mechanistic studies demonstrating how the supply of multiple essential nutrients modulates decomposition of plant remains due to changes in microbial activity are lacking.

To better predict the outcomes of interactions between nutrient enrichment and C cycling, we addressed the following questions across twenty-one grasslands around the globe that are part of the Nutrient Network research cooperative (NutNet): (i) How does nutrient addition (N, P, and K plus nine essential macro- and micronutrients [hereafter, K+μ]) affect decomposition rates and further biochemical stabilization of buried standard plant materials, *sensu* Keuskamp *et al.* (2013)? (ii) How does nutrient addition alter the extracellular enzyme activity of microbial communities and how does this, in turn, affect initial decomposition rates and biochemical stabilization of plant remains? How does between-site climate variability affect plant matter

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123 decomposition, stabilization and microbial activity and how does it interact with the addition of
124 multiple essential nutrients? (iii) How do changes in initial decomposition rates in response to
125 nutrient addition covary with observed changes in biochemical stabilization of plant remains?

126 Based on previous experimental evidence from local and regional NutNet studies on soil
127 organic matter dynamics (Riggs *et al.*, 2015; Crowther *et al.*, 2019), the high amounts of
128 nutrients added (10 g m^{-2} yr^{-1}) (Knorr *et al.*, 2005), and the short-term nature of our incubations
129 (i.e., ninety days) (Berg, 2014), we hypothesised that initial decomposition rates would increase
130 in nutrient addition plots, particularly in those receiving the full suite of nutrients (Knorr *et al.*,
131 2005; Berg, 2014). Given that microbial communities largely drive nutrient cycling through the
132 release of extracellular enzymes (Sinsabaugh *et al.*, 2009), we also predicted that the effects of
133 nutrient addition on the decomposition of buried plant remains would be accompanied by an
134 increase in the enzymatic potential of soil microbial communities, with which plant remains were
135 in close contact. Globally coordinated experiments like the one presented here are essential to
136 predict the biogeography of microbial processing potential of plant materials under global
137 change. They may also help to improve the outcome of Earth system models by helping to
138 constrain parameters for microbial activity under future scenarios of global environmental
139 change (Allison, 2012).

140

141 **METHODS**

142 This study was carried out in twenty-one globally distributed grasslands that are part of the
143 Nutrient Network (www.nutnet.org). Sites included a wide range of grassland types: tundra
144 grasslands, annual grasslands, mesic grasslands, montane meadows, old fields, semiarid

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grasslands, shortgrass prairies, tallgrass prairies and Mediterranean grasslands. Sites are located in North and South America, Europe and Oceania and span wide ranges of mean annual precipitation (203–1507 mm yr^{-1}), mean annual temperature (-3.2–23.7 °C) and latitude (52°S–69°N, Supplementary Figure 1 and Supplementary Table 1). We focused on grasslands because they cover a large proportion of the global land surface (approximately 40%), span a range of environmental gradients, serve as a source of forage and food production and sequester large amounts of organic C (Conant *et al.*, 2001; O'Mara, 2012).

Each site consists of a full factorial combination of N, P, and K plus nine essential macro- and micronutrient (K+μ) additions, typically with three (and up to five) replicates per treatment and site, in a randomized block design (Borer *et al.*, 2014; Hautier *et al.*, 2014). Essential macro- and micronutrients added alongside with K were calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), Mn, molybdenum (Mo), and zinc (Zn). Nutrients are added at a rate of 10 g N m^{-2} yr^{-1} as timed-release urea, 10 g P m^{-2} yr^{-1} as triple-super phosphate, 10 g K m^{-2} yr^{-1} as potassium sulfate and 100 g m^{-2} yr^{-1} of a micronutrient mix (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn). Nitrogen, P, and K are applied annually, whereas the micronutrient mix was applied only once in the beginning. Each plot is 5 x 5 m and is divided into four 2.5 x 2.5 m subplots. Each subplot is further divided into four 1 x 1 m square sampling plots, one of which is set aside for soil sampling. Plots are separated by at least 1-m wide walkways.

Decomposition of buried plant remains

At each site, we assessed decomposition rates and stabilization of buried plant remains using the Tea Bag Index (TBI) (Keuskamp *et al.*, 2013). The TBI is a method for evaluating plant matter decomposition that uses two types of commercially available tea bags (green tea [labile

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substrate] and rooibos [recalcitrant substrate]) as standardized test kits over a 90-day incubation period. The TBI uses the relative loss of tea mass to calculate metrics of (i) the decomposition rate (*k*) and (ii) a stabilization factor (*S*). The stabilization factor essentially quantifies the degree to which the labile fraction of green tea remains (i.e., it is stabilized) during later phases of the process, where decomposition rates are assumed to be negligible, and has been shown to correlate with soil C sequestration suitability (Keuskamp *et al.*, 2013). Although green tea and rooibos do not by any means accurately represent the real quality of superficial and buried dead plant remains across the studied grasslands, the TBI has been shown to adequately characterize the decomposition environment by measuring its potential to decompose and stabilize the deployed standardized material (Mueller *et al.*, 2018), thus providing standardized indices of early and later phases in the decomposition process that can be compared across sites and treatments (Keuskamp *et al.*, 2013). Pros and limitations of this method and other similar ones such as the burial of cotton and cellulose strips have been extensively presented and discussed in previous studies (Clark, 1970; Risch *et al.*, 2007; Mueller *et al.*, 2018), and are thus not discussed here.

Between two and four pairs of green tea (product barcode number: 8722700055525) and rooibos tea (product barcode number: 8722700188438) bags per plot were buried at each site (8 cm depth) for ~90 days according to the protocol extensively described in Keuskamp *et al.* (2013) and in www.teatime4science.org. After the incubation period, tea bags were collected and cleaned by hand (no water used). One/two of the pairs were oven-dried at 60 °C for 48 h and then weighed to determine *k* and *S*, whereas the other one/two pairs were immediately frozen at -20 °C. Decomposed frozen samples were shipped as cooled as possible to Madrid, Spain, where they were used to carry out microbial extracellular enzyme activity assays.

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Enzyme assays

Partially decomposed samples were assayed for seven enzymes related to the main biogeochemical nutrient cycles: (i) C-cycle enzymes: α- and β-1,4-glucosidase (AG, BG; EC 3.2.1.20 and EC 3.2.1.21), xylosidase (XYL; EC 3.2.1.37), and β-D-cellobiohydrolase (CB; EC 3.2.1.91) enzymes, involved in the degradation of starch, cellulose and other alpha- and beta-linked glucans (the major components of plant cell walls); (ii) N-cycle enzymes: β-1,4-N-acetylglucosaminidase (NAG; EC 3.2.1.14), associated with the degradation of chitin and peptidoglycans (major microbial cell wall components) and leucine aminopeptidase (LAP; EC 3.4.11.1), which catalyzes the hydrolysis of leucine residues at the N-terminus of peptides and proteins and; (iii) P-cycle enzymes: acid phosphatase (PHOS; phosphorus mineralization; EC 3.1.3.2). Briefly, assays were conducted by homogenizing ~0.5 g of frozen decomposed plant remains in 30 mL of pH-adjusted 50 mM sodium acetate buffer to match the pH of tea (4.75 on average for both teas). The homogenized solutions were then added to black, flat-bottomed 96-well plates. Replicate decomposed plant matter slurry controls and 4-methylumbellfferone (MUB) standard curves of 0-100 µm were included in each sample. Fluorometric substrates (Sigma-Aldrich, reference numbers: M9766 for AG, M3633 for BG, M7008 for XYL, M6018 for CBH, M2133 for NAG, L2145 for LAP, and M8883 for PHOS) were added to slurries and then incubated for 1.5 h at 35 °C. Following incubation, the plates were scanned on a microplate fluorometer (Synergy HTX) using an excitation wavelength of 365 nm and an emission wavelength of 450 nm.

Statistical analyses

All statistical analyses were carried out in R v3.6.0. The effects of nutrient addition on plant matter decomposition parameters (*k* and *S*) and enzyme activity were analyzed in a linear mixed

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effects model framework using the 'lme' function from the *nlme* package, with N, P and K as fixed factors (full model, including all possible interactions) nested within experimental sites (random factor).

We then used Spearman Rank correlations to explore relationships among decomposition parameters, all individual enzyme activities and bioclimatic drivers extracted from WorldClim (Fick & Hijmans, 2017). Information obtained from these relationships as well as from *a priori* knowledge was used to develop a conceptual model that could be subsequently tested using structural equation modeling (Grace, 2006). In our *a priori* model, we included distance to equator to account for potential spatial effects and the role of unobserved variables that may vary across large geographical gradients. Distance to equator was predicted to affect all variables, except the experimental treatments, which were predicted to influence microbial activity and decomposition and stabilization of plant remains. Distance to equator was included to account for spatial effects associated with unmeasured variables that could be important in driving the decomposition response. Based on our results (see below), we did not include interactions among nutrients, but considered interactions between nutrient additions and climate. Climate drivers included in the analysis were mean annual precipitation and temperature of the wettest quarter, also based on our results. In our conceptual model, microbial activity was predicted to affect k and S. Initial decomposition was, in turn, considered as a predictor of the stabilization factor to account for the current thinking about soil organic matter formation after decomposition of labile fractions of plant remains and accumulation of by-products of microbial metabolism and dead cells (Cotrufo *et al.*, 2013). We assumed that precipitation and temperature of the wettest quarter, on one hand, and microbial enzymes measured on the green and rooibos tea, on the other hand, would covary, but do not infer causal relationships; thus, they were modelled using correlated

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error terms. Finally, we included microbial enzymes related to N mineralization over other microbial enzymes related to C and P because, despite all enzymes being highly multi-correlated, N-related enzymes showed the clearest patterns. To test this model, we followed a d-sep approach using the *piecewiseSEM* package (version 2.0.2), in which a set of linear structured equations are evaluated individually. This approach allows us to account for nested experimental designs. To run the linear mixed models, we used the lme function of the nlme package, including site as a random factor. Good fit was assumed when Fisher's C values were non-significant ($p > 0.05$).

RESULTS

Initial decomposition rates (*k*) of buried plant remains increased by 37% and 72% with NP and NPK+μ additions, respectively (Figure 1). Initial decomposition rates (*k*) of buried plant remains also weakly increased with N ($F_{1,472} = 10.81$; $P = 0.001$; 17.3% increase) and P addition ($F_{1,472} = 6.90$; $P = 0.009$; 15.4% increase) across our 21 grasslands when either all N or P treatment combinations were considered together (Supplementary Figures 2-3). We found no significant interactions (all $P > 0.1$) and observed no effect of K+μ addition ($F_{1,472} = 1.44$; $P = 0.230$). Similarly, the stabilization factor (*S*) of buried plant remains increased by 12% and 16% with NP and NPK+μ additions, respectively (Figure 1). The stabilization factor (*S*) was also higher with N ($F_{1,475} = 3.74$; $P = 0.054$; marginal effect) and P addition ($F_{1,475} = 7.88$; $P = 0.005$) when either all N or P treatment combinations were considered together (Supplementary Figures 2-3), but not with K+μ addition ($F_{1,475} = 2.32$; $P = 0.129$) (Fig. 1b, Supplementary Figure 2). Again, no interactions were found (all $P > 0.1$).

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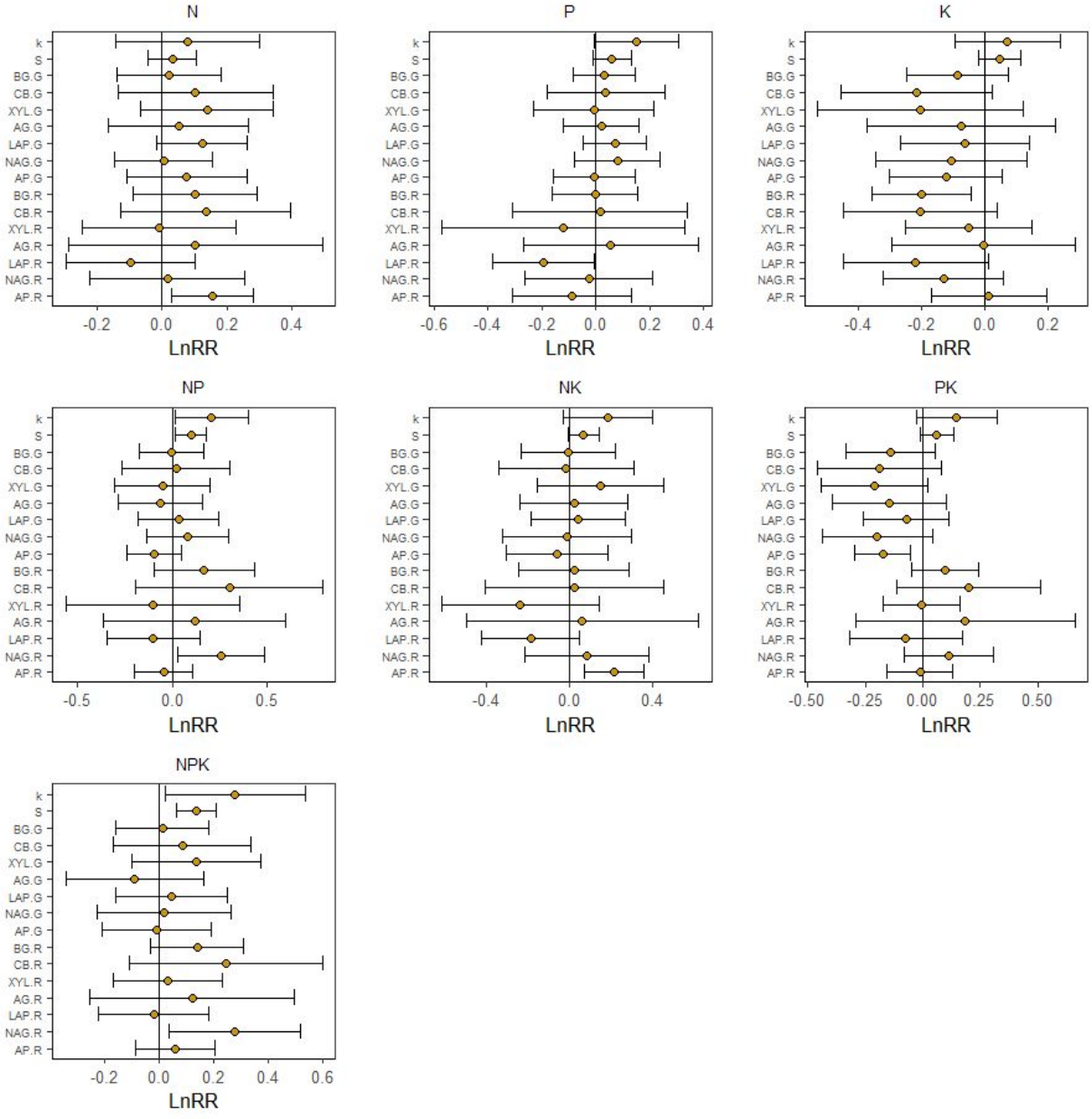
Figure 1. Nutrient addition effects on initial decomposition rate (*k*) and stabilisation factor (*S*) of plant remains and microbial exoenzymes related to the main biogeochemical cycles (C, N and P) measured on the partially decomposed green tea (G) and rooibos (R) substrates. BG = ß-glucosidase. CB = cellobiohydrolase. AG = α-glucosidase. XYL = xylosidase. NAG = N-acetyl-glucosaminidase. LAP = leucine aminopeptidase. AP = acid phosphatase. LnRR = natural logarithm of the response ratio ($variable_{treatment}/variable_{control}$). Error bars are 95% confidence intervals of the response across experimental sites and treatments. Error bars that do not cross the zero line are deemed as significant ($P < 0.05$).



Microbial enzyme activities involved in the C, N and P biogeochemical cycles differed across substrate types. Green tea remains, originally consisting mostly of labile substrate(Keuskamp *et al.*, 2013), had greater C-related ($F_{1,716}$ = 357.7; P < 0.001), N-related ($F_{1,716}$ = 293.0; P < 0.001) and P-related ($F_{1,716}$ = 7.48; P = 0.006) microbial enzyme activity rates compared to rooibos remains that initially consisted mostly of recalcitrant substrate(Keuskamp *et al.*, 2013) (Supplementary Figure 4). Moreover, nutrient addition affected potential microbial enzyme activity measured on the decomposed plant remains, although the effects were larger and more common for more recalcitrant rooibos remains. The addition of NP and NPK increased N-acetyl-glucosaminidase activity, an enzyme involved in N mineralization, measured on rooibos tea by 42% and 45%, respectively (Figure 1 and Supplementary Figure 5), while adding N or NK increased phosphatase activity by 20% and 28%, respectively (Figure 1). Nitrogen addition weakly but consistently increased the activity of C-, N- and P-related enzymes measured on rooibos tea (C enzymes: $F_{1,342}$ = 5.20; P = 0.023; N enzymes: $F_{1,342}$ = 6.00; P = 0.015; P enzymes: $F_{1,342}$ = 6.56, P = 0.011) when all N treatment combinations were considered simultaneously (Supplementary Figures 4 and 6). When all treatment combinations were considered simultaneously, P additions weakly increased microbial C- and N-related activity (C enzymes: $F_{1,342}$ = 3.01; P = 0.084; N enzymes: $F_{1,291}$ = 3.51; P = 0.062; Supplementary Figures 4-6), and decreased P-related activity ($F_{1,291}$ = 3.29; P = 0.070; Supplementary Fig. 5) in the rooibos tea. More in-depth exploration showed that the main differences in phosphatase activity were between the P-only and NK+μ treatments (Tukey test: z-value = -3.00; P = 0.055), indicating the usefulness of phosphatase activities measured on decomposed plant remains as a reliable indicator of P limitation across global grasslands. In contrast, K+μ additions reduced ß-glucosidase activity measured on green tea (Figure 1). Moreover, when all treatment

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combinations were considered simultaneously, K+μ additions reduced the activity of C- and N-related enzymes on the green tea substrate (C enzymes: $F_{1,346} = 3.60$; $P = 0.059$; N enzymes: $F_{1,346} = 6.27$; $P = 0.013$; all K+μ treatment combinations included).

Although nutrient addition generally increased decomposition of buried plant remains across our study sites, decomposition and effects sizes in response to treatments varied between- and within-sites (Figs. 2 and 3; Supplementary Figures 7-9). For example, the decomposition rate (k) decreased with increasing N-related microbial enzyme activity measured on green tea substrate and temperature of the wettest quarter (Fig. 2 and Supplementary Figures 7 and 8) and increased with mean annual precipitation (Supplementary Figure 8) and N-related microbial enzyme activity measured on rooibos substrate (Fig. 2). The stabilization factor was positively related to N-related microbial activities measured on rooibos substrate and negatively related to temperature of the wettest quarter (Fig. 2 and Supplementary Figures 7 and 8). Moreover, the addition of nutrients significantly altered the strength of some decomposition relationships with climatic conditions and microbial enzymes. Decomposition rates among sites were associated with a steeper negative relationship between temperature of the wettest quarter and the stabilization factor under P additions (interaction: $P = 0.010$) (Fig. 2). We also found a steeper negative relationship between temperature of the wettest quarter and the decomposition rate under N additions (interaction: $P = 0.022$), while the increased decomposition rates with elevated P supply was conditional on higher rainfall (interaction: $P = 0.016$) (Fig. 2 and Supplementary Figure 9).

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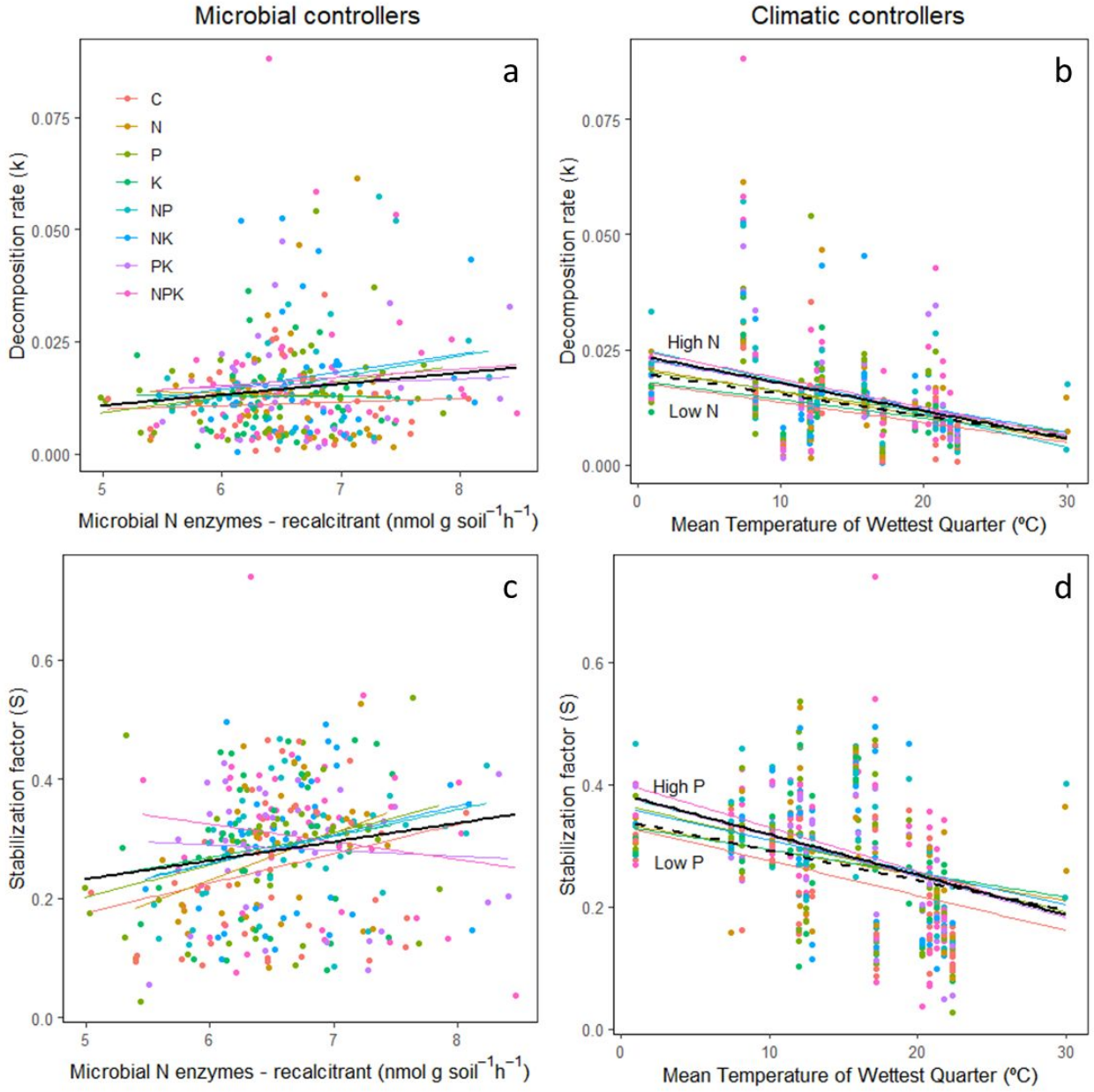
Figure 2. Relationships between initial decomposition rate (*k*) and stabilisation factor (*S*) and their most relevant microbial (a, c) and climatic (b, d) controllers based on correlations presented in Supplementary Figure 5. Data points are means for each plot (n = 500 for *k*; n = 503 for *S*). Solid line in panels (a) and (c) is the fitted linear model across all plots. In panel (b), dashed and solid lines are the fitted linear models under control and high-N conditions, respectively. In panel (d), dashed and solid lines are the fitted linear models under control and high-P conditions, respectively. Color lines based on the legend in panel (a) are the fitted linear models for each experimental treatment. Enzyme activities are log-transformed. Relationships under ambient-only conditions are shown in Supplementary Figure 6.



Finally, our SEM explained 28% of *k* variability and 25% of *S* variability (Fig. 3). We found that the consistent positive effects of N and P addition on *k* and *S* were indirect and occurred mainly through the effects of N and P additions on the activity of microbial enzymes related to N mineralization measured on the recalcitrant fractions of buried plant remains. Overall, the positive effect of fertilization was most important in wetter (*k*) and colder climates during the wet season (*k* and *S*). In addition, K+μ addition had a positive but weak effect on stabilization of plant remains.

DISCUSSION

Our results demonstrate that the acceleration of initial decomposition rates (*k*) and decreased decomposition in the later phase (*S*) are likely widespread phenomena in response to soil nutrient enrichment, independent of the origin and chemical composition of the plant remains. These results are in agreement with previous meta-analyses that showed that N addition of 7.5–12.5 g N m^{-2} y^{-1} enhanced decomposition rates across ecosystems (Knorr *et al.*, 2005; Zhang *et al.*, 2018). Moreover, our results also provide the first empirical evidence that microbial decomposition of unprotected plant matter is primarily limited by N and P availability (Sinsabaugh *et al.*, 1993; Hobbie & Vitousek, 2000), but show that other essential nutrients are also relevant drivers of plant matter decomposition at the global scale (Kaspari *et al.*, 2008b; Keiluweit *et al.*, 2015; Kaspari & Powers, 2016; Ochoa-Hueso *et al.*, 2019b), and thus should not be overlooked. However, our results also indicate a very limited potential for nutrient management to alter soil C cycling in global grasslands, due to the moderate magnitude of the effect and its great variability across sites.

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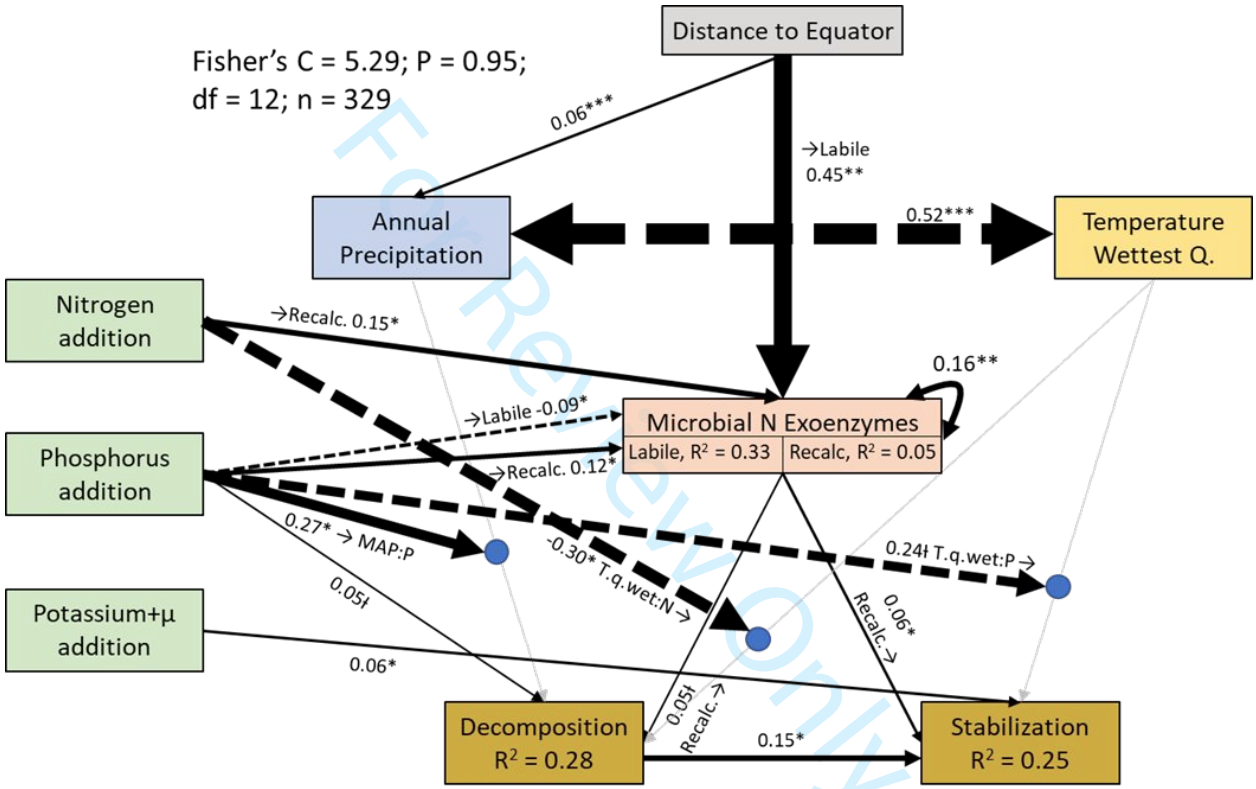
Figure 3. Structural equation model. Solid lines = positive associations. Dashed lines = negative association. Line width is proportional to the strength of the association. Bidirectional arrows indicate variables with correlated error terms, but do not necesarilly imply causality. ***P < 0.001; **P < 0.01; *P < 0.05; †P < 0.1. Microbial N exoenzymes = microbial enzymes related to the N biogeochemical cycle measured on the decomposed green tea (labile) and rooibos substrates (recalcitrant). The full a-priori conceptual model (i.e., with non-significant paths included) can be found in Supplementary Figure 10. Arrowheads pointing to blue dots indicate significant interaction terms.



Despite these commonalities in the response of decomposition to nutrients, decomposition of buried plant remains varied widely across our study sites likely due to variations in local climatic conditions and the metabolic toolkit of soil microbial communities to process plant remains. For example, our results of greater decomposition and stabilization under lower temperature of the wettest quarter is in agreement with a global study of tidal wetlands that showed greater stabilization potential with lower temperatures (Mueller *et al.*, 2018) and with the

362 greater known potential of colder and moister, northern biomes to store more C in the soil (Plaza
363 *et al.*, 2018). This also coincides with the previously reported negative relationship between
364 temperature of the wettest quarter and laboratory net N mineralization in global grasslands, but
365 contrasts with the positive relationship found between the two when soil incubations were
366 carried out in the field (Risch *et al.*, 2019). Moreover, the addition of nutrients significantly
367 altered the strength of some decomposition relationships with climatic conditions and microbial
368 enzymes. These results further demonstrate widespread co-limitation of decomposition by the
369 availability of water and multiple essential nutrients; factors that are also important for plant
370 productivity and soil C capture in global grasslands (Eskelinen & Harrison, 2015; Crowther *et*
371 *al.*, 2019). These results highlight the strong coupling between multiple nutrient limitation, soil
372 eutrophication and climatic factors, with likely consequences for the global C cycle under future
373 fertilization regimes/nutrient pollution scenarios and warmer and drier climates (Falkowski *et al.*,
374 2000).

375 Microbial activity was consistently higher in the labile substrate, which likely reflects the
376 greater ability of soil microbial communities to quickly colonise and decompose more labile
377 substrates due to their higher nutritional quality (Chapin *et al.*, 2002). Moreover, the
378 downregulation of microbial activity under K+μ additions suggests that the release of some of
379 these enzymes may be associated with the mining of other essential macro- and micronutrients
380 from labile organic substrates when these are in short supply. These results show that the
381 metabolic expression of microbial communities differed across the experimental treatments and
382 plant matter substrates, likely due to changes in the composition and abundance of soil bacterial
383 and fungal communities, as described before (Allison, 2012; Leff *et al.*, 2015). These results also
384 suggest that shifts in the composition of plant communities and associated changes in the quality

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385 of their dead matter inputs due to eutrophication may further alter the functioning of soil
386 microbial communities (Bradford *et al.*, 2016; Bjorkman *et al.*, 2018).

387 Finally, we sought to gain an ecosystem-level understanding of climatic and microbial
388 drivers of *k* and *S* under soil eutrophication across global grasslands, for which we used
389 structural equation modelling. Our results are among the first empirical indication of the ability
390 of microbial communities to mineralize N from recalcitrant plant fractions as a determinant of
391 greater *k* and *S* under eutrophication scenarios in global grasslands, particularly in alpine and
392 boreal ecosystems. Moreover, *k* was also positively related to stabilization rates, suggesting that
393 faster initial decomposition is compatible with disproportionately larger accumulation of slowly
394 decomposing, highly transformed plant remains during later phases. This is possibly linked with
395 the more efficient stabilization of microbial waste products generated during the fast break-down
396 and consumption of plant remains by microbes (Cotrufo *et al.*, 2013; Lange *et al.*, 2015; Riggs *et*
397 *al.*, 2015). An alternative explanation is that microbes that are good at decomposing plant
398 remains quickly, target material that is easily degradable and outcompete those microbes that
399 could decompose more complex C, thereby leaving a high proportion of undecomposed material
400 that is eventually biochemically stabilized.

401 Taken together, our results demonstrate that the microbial decomposition of buried plant
402 remains is weakly but consistently co-limited by the availability of multiple essential macro- and
403 micronutrients in grasslands worldwide and will respond interactively to climate variations and
404 soil eutrophication, at least during the initial phases. Although adding limiting nutrients to
405 managed grasslands may thus appear as a viable strategy to enhance soil C cycling and perhaps,
406 ultimately, increase soil C sequestration via greater stabilization of physically unprotected plant
407 remains that are in close contact with the soil (Conant *et al.*, 2017), our results also imply that the

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outcomes of these efforts may be very weak and hampered by global warming and the increased frequency of drought events. This climatic dependency, the high monetary costs of this operation, its operational unfeasibility, and the known widespread negative consequences of adding mineral fertilizers and increased N deposition for above and belowground grassland biodiversity (Borer *et al.*, 2014; Harpole *et al.*, 2016; Hautier *et al.*, 2018), suggest that the environmental and economic costs of soil eutrophication in grasslands may be disproportionally higher than any potential positive effects due to enhanced decomposition and stabilization of plant remains.

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