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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 aggregate-unprotected organic matter inputs is key for soil fertility, long-term ecosystem carbon and nutrient sequestration and sustainable agriculture. We investigated the effects of adding multiple nutrients (nitrogen, phosphorus and potassium plus nine essential macro- and micro-nutrients) on decomposition and biochemical transformation of standard plant materials buried in 21 grasslands from four continents. Addition of multiple nutrients weakly but consistently increased decomposition and biochemical transformation of plant remains during the peak-season, concurrent with changes in microbial exoenzymatic activity. Higher mean annual precipitation and lower mean annual temperature were the main climatic drivers of higher decomposition rates, while biochemical transformation of plant remains was negatively related to temperature of the wettest quarter. Nutrients enhanced decomposition most at cool, high rainfall sites, indicating that in a warmer and drier future fertilized grassland soils will have an even more limited potential for microbial processing of plant remains.

KEYWORDS
carbon cycling and sequestration, decomposition, eutrophication, fertilization, microbial activity, NutNet, nutrient (co-)limitation

1 | INTRODUCTION

Many ecosystems worldwide are receiving greater inputs of readily available nutrients due to increasing contributions from various anthropogenic sources (Fowler et al., 2013; Sala et al., 2000). For example, many grasslands are fertilized with nitrogen (N), phosphorus (P), potassium (K) and other essential macro- and micro-nutrients to improve pasture yield and nutritional quality (Conant, Paustian, & Elliot, 2001). Additionally, the non-intentional atmospheric and aeolian deposition of biologically limiting nutrients is a common source of eutrophication in these ecosystems (Fowler et al., 2013; Gruber & Galloway, 2008). Considered as a whole, natural, seminatural and anthropogenic grasslands cover a large proportion of the global land surface (~40%), serve as a source of forage and food production and store approximately 20%–30% of all terrestrial carbon (C), most of it in the soil (Conant et al., 2001; O'Mara, 2012; Scurlock & Hall, 1998). The rate of decomposition and biochemical transformation of superficial and buried aggregate-unprotected plant remains is a lynchpin for soil fertility and ecosystem-level C fluxes in grassland ecosystems, hence for their sustainability (Bradford, Berg, Maynard, Wieder, & Wood, 2016; Cadisch & Giller, 1997). 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.

Breakdown 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 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 down, stabilizing at a limit value (Berg, De Santo, Rutigliano, Fierro, & Ekbohm, 2003), as labile compounds are lost or transformed to recalcitrant compounds that accumulate together with microbial necromass (Bradford et al., 2016). Soil microbial communities play an important role in these processes as they release extracellular enzymes that breakdown different types of plant materials (Leff et al., 2015; Philippot, Raaijmakers, Lemanceau, & van der Putten, 2013; 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 their relative proportions of labile and recalcitrant fractions decompose (Wickings, Grandy, Reed, & Cleveland, 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; Kaspari et al., 2008; Kaspari, Yanoviak, Dudley, Yuan, & Clay, 2009; Keiluweit et al., 2015; Knorr, Frey, & Curtis, 2005; Ochoa-Hueso et al., 2019) 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 soil nutrient enrichment and microbial processing of aggregate-unprotected plant remains in soil, we addressed the following questions across 21 grasslands around the globe that are part of the Nutrient Network research cooperative (NutNet): (a) How does nutrient addition (N, P, and K plus nine essential macro- and micronutrients [hereafter, K + μ]) affect decomposition rates and further biochemical transformation of buried standard plant materials? (b) How does nutrient addition alter the extracellular enzyme activity of microbial communities and does this, in turn, affect initial decomposition rates and biochemical transformation of plant remains? (c) How does among-site climate variability affect plant matter decomposition and microbial activity and how does it interact with the addition of multiple essential nutrients? (d) How do changes in initial decomposition rates in response to nutrient addition covary with observed changes in biochemical transformation of plant remains?

Based on previous experimental evidence from local and regional NutNet studies on soil organic matter dynamics (Crowther et al., 2019; Riggs, Hobbie, Bach, Hofmockel, & Kazanski, 2015) and the high amounts of nutrients added (10 g m^{-2} $year^{-1}$; Knorr et al., 2005), we hypothesized that, over short-term incubations (i.e. 90 days; Berg, 2014), early decomposition rates would increase in nutrient addition plots, particularly in those receiving the full suite of nutrients (Berg, 2014; Knorr et al., 2005). Given that microbial communities largely drive nutrient cycling through the release of extracellular enzymes (Sinsabaugh, Hill, & Follstad Shah, 2009), we also predicted that the effects of nutrient addition on the decomposition of buried plant remains would be accompanied by an increase in the enzymatic potential of soil microbial communities, with which plant remains were in close contact. We additionally expected that short-term decomposition would be more rapid at sites with a higher mean annual precipitation (Austin & Vitousek, 2000). Globally coordinated experiments like the one presented here are essential to predict the biogeography of microbial processing potential of plant materials under global change. They may also help to improve the outcome of Earth system models by helping to constrain parameters for microbial activity under future scenarios of global environmental change (Allison, 2012; Luo et al., 2016; Wieder et al., 2015).

2 | METHODS

This study was carried out in 21 globally distributed grasslands that are part of the Nutrient Network (www.nutnet.org; Borer, Harpole, et al., 2014). Sites included a wide range of grassland types: tundra grasslands, annual grasslands, mesic grasslands, montane meadows, old fields, semiarid 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 (203–1,507 mm/year), mean annual temperature (−3.2 to 23.7°C) and latitude (52°S–69°N, Figure S1; Table S1).

Each local experimental set-up consists of a full factorial combination of N, P and K plus nine essential macro- and micro-nutrient (K + μ) additions, typically with three (and up to five) replicates per treatment and site, in a randomized block design (Borer, Grace, Harpole, MacDougall, & Seabloom, 2017; Borer, Harpole, et al., 2014; Hautier et al., 2014). Essential secondary macro- and micro-nutrients 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} $year^{-1}$ as timed-release urea, 10 g P m^{-2} $year^{-1}$ as triple-super phosphate, 10 g K m^{-2} $year^{-1}$ as potassium sulfate and 100 g m^{-2} $year^{-1}$ of a macro- and micro-nutrient 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 nutrient mix was applied only once in the beginning. Each plot is 5 × 5 m and is divided into four 2.5 × 2.5 m subplots. Each subplot is further divided into four 1 × 1 m square sampling plots, one of which is set aside for soil sampling. Plots are separated by at least 1 m wide walkways.

2.1 | Decomposition of buried plant remains

At each site, we assessed decomposition rates and biochemical transformation of buried plant remains using the Tea Bag Index (TBI; Keuskamp, Dingemans, Lehtinen, Sarneel, & Hefting, 2013). The TBI is a method for evaluating plant matter decomposition that uses two types of commercially available tea bags (green tea [more labile substrate] and rooibos [more 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 (a) the decomposition rate (*k*) and (b) a stabilization factor (*S*). The stabilization factor essentially quantifies the proportion of green tea that remains during later phases of the process, where decomposition rates are assumed to be negligible. The *S* factor has been suggested to correlate with soil C storage suitability (Keuskamp et al., 2013). However, due to absence of physical interaction of the substrate with soil minerals, we interpret it more as an index of biochemical transformation of the green tea substrate, as opposed to the substrates being respired and their C lost to the atmosphere. Moreover, although green tea and rooibos tea do not 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 biochemically transform the deployed standardized material (Mueller et al., 2018). Thus, it provides standardized indices of early and later phases in the decomposition process

that are critical for direct comparisons across sites and treatments (Keuskamp et al., 2013). Benefits and limitations of this and other similar methods, such as the burial of cotton and cellulose strips, have been extensively presented and discussed elsewhere (Clark, 1970; Mueller et al., 2018; Risch, Jurgensen, & Frank, 2007). The main limitations include impeding fragmentation by soil fauna and the transfer of residue fragments into the mineral soil, which contribute to the formation of particulate organic matter (Cotrufo, Wallenstein, Boot, Denef, & Paul, 2013).

Between two and four pairs of green tea (product barcode number: 8722700055525) and rooibos tea (product barcode number: 8722700188438) pyramid-shaped nylon mesh bags were buried per plot at each site (8 cm depth) for ~90 days. 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 hr and then weighed to determine *k* and *S*, whereas the other one/two pairs were immediately frozen at −20°C. Frozen samples were shipped as cooled as possible to the Autonomous University of Madrid, Spain, where they were used to carry out microbial extracellular enzyme activity assays.

2.2 | Enzyme assays

Partially decomposed samples were assayed for seven enzymes related to the main biogeochemical nutrient cycles: (a) C cycle enzymes: α- and β-1,4-glucosidase (AG and 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; (b) 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 catalyses the hydrolysis of leucine residues at the N-terminus of peptides and proteins; and (c) P-cycle enzymes: acid phosphatase (PHOS; phosphorus mineralization; EC 3.1.3.2). Prior to analyses, decomposed plant remains were carefully extracted from the nylon bags, avoiding contamination with residues attached to the external part of the bags. Soils were not able to penetrate inside the bags, which means that analyses were consistently done on decomposed plant remains. Briefly, assays were conducted by homogenizing ~0.5 g of frozen and 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 hr 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.

2.3 | Statistical analyses

All statistical analyses were carried out in R v3.6.0. The effects of nutrient addition on decomposition parameters (*k* and *S*) of plant remains and enzyme activity were analysed using the natural logarithm of response ratios, defined as ($variable_{treatment}/variable_{control}$) and in a linear mixed 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 also used linear mixed models to explore relationships among decomposition parameters, all individual enzyme activities and bioclimatic drivers extracted from WorldClim (Fick & Hijmans, 2017).

A priori knowledge was used to develop a conceptual model that could be subsequently tested using structural equation modelling (Grace, 2006). Results obtained from mixed models were used to fine-tune our variable selection, for example, by showing which climatic variables best explained microbial enzyme activity and decomposition. 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, climate and experimental treatments were predicted to influence microbial enzyme activity and decomposition and biochemical transformation of plant remains. Based on our own results, we did not include interactions among nutrients in our model, but we considered interactions between nutrient additions and climate. Climate drivers included in the analysis were mean annual precipitation, mean annual temperature and temperature of the wettest quarter. In our conceptual model, microbial activity was predicted to affect *k* and *S*. Decomposition rate *k* was, in turn, considered as a predictor of the stabilization factor *S*. We did so because *S* is assumed to represent the proportion of biochemically transformed plant residues that remain during the later phases of the decomposition process, while *k* provides a standardized index of the decomposition rate during the early phase. This framework is compatible with the importance of biochemical transformation of labile fractions of plant remains and accumulation of by-products of microbial metabolism and dead cells for soil organic matter formation during the decomposition process (Cotrufo et al., 2013). We assumed that distance to equator and climatic variables, on one hand, and microbial enzymes measured on the green and rooibos tea, on the other hand, would covary; thus, they were modelled using correlated error terms. Finally, we included microbial enzymes related to N mineralization over other microbial enzymes related to C and P because, although all enzymes were 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 allowed us to account for nested experimental designs. To

run the individual linear mixed models for the SEM, we used the 'lme' function of the *nlme* package, including site as a random factor, as previously explained. We used non-significant ($p > .05$) Fisher's *C* values to indicate good fit.

3 | RESULTS

Initial decomposition rates (*k*) of buried plant remains increased by 35%, 41%, 43% and 79% with P, NP, NK + μ, and NPK + μ additions respectively (Figures 1a and 2; Figure S3). Initial decomposition rates (*k*) of buried plant remains also weakly increased with N ($F_{1,496} = 14.06$; $p < .001$) and P addition ($F_{1,496} = 8.49$; $p = .004$) across our 21 grasslands when either all N or P treatment combinations were considered together (Table S2; Figure S4). We found no significant interactions (all $p > .1$) and observed no effect of K + μ addition only ($F_{1,496} = 1.57$; $p = .211$). The stabilization factor (*S*) of buried plant remains increased between 14% and 22% in response to all nutrient treatments, except for the N-only treatment (Figures 1b and 2; Figure S5). The stabilization factor (*S*) was also higher with N ($F_{1,528} = 4.17$; $p = .042$) and P addition ($F_{1,528} = 12.72$; $p < .001$) when either all N or P treatment combinations were considered together, but not with K + μ addition ($F_{1,528} = 2.44$; $p = .118$; Table S2; Figure S4). Results regarding the raw mass loss data, used to calculate the TBI parameters, revealed that the mass loss of green tea decreased between 3% and 6% in response to all nutrient combination treatments, except for the N-only treatment (Figures 1b,c and 2). The mass loss of green tea decreased with P addition ($F_{1,530} = 15.14$; $p < .001$), while the mass loss of rooibos tea increased with N addition ($F_{1,533} = 8.44$; $p = .004$) when all N or P treatment combinations were considered together (Table S2).

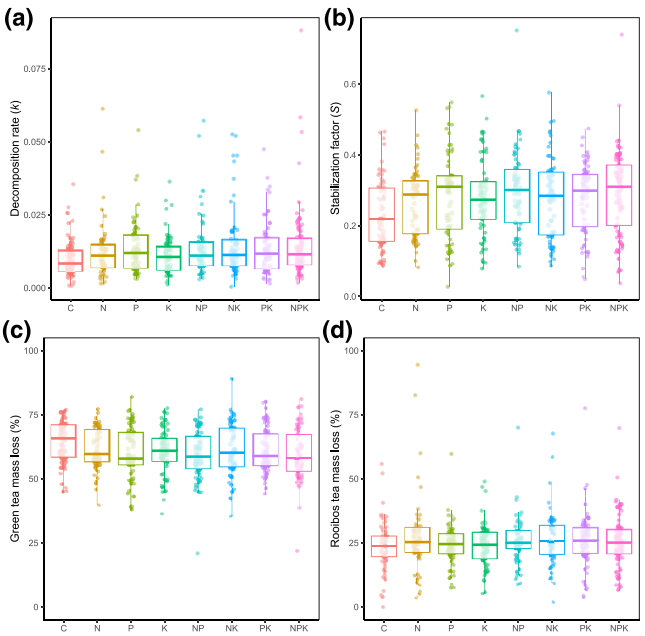

FIGURE 1 Boxplots of nutrient addition effects on (a) initial decomposition rate (*k*), (b) stabilization factor (*S*) and mass loss of (c) green tea and (d) rooibos. Median and first and third quartile are shown. *k* is indicative of decomposition of plant remains and is based on green tea and rooibos tea, whereas *S* is indicative of labile compounds that are biochemically transformed during the late phase of the decomposition process. C, control; K, potassium + micronutrients; N, nitrogen; P, phosphorus. Data points are means for each plot. Detailed results from linear mixed effects models are provided in Table S2 [Colour figure can be viewed at wileyonlinelibrary.com]

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 (84%; Keuskamp et al., 2013), had greater C-related ($F_{1,716} = 357.7$; $p < .001$), N-related ($F_{1,716} = 293.0$; $p < .001$) and P-related ($F_{1,716} = 7.48$; $p = .006$) microbial enzyme activity rates compared to rooibos remains that had a greater proportion of recalcitrant substrate (45%; Keuskamp et al., 2013; Figure 3; Figures S6–S13). Moreover, nutrient addition affected potential microbial enzyme activity measured on the decomposed plant remains, although the effects were larger and more common for the more recalcitrant rooibos remains (Figures 2 and 3; Figures S6–S13). The addition of NP and NPK + μ increased *N*-acetyl-glucosaminidase activity, an enzyme involved in N mineralization, measured on rooibos tea by 42% and 45%, respectively (Figure 2f,g), while adding N or NK + μ increased phosphatase activity measured on the rooibos tea by 20% and 28%, respectively (Figures 2a,f and 3f). Nitrogen addition weakly but consistently increased the activity of C-, N- and P-related enzymes measured on rooibos tea (C enzymes: $F_{1,342} = 5.20$; $p = .023$; N enzymes: $F_{1,342} = 6.00$; $p = .015$; P enzymes: $F_{1,342} = 6.56$, $p = .011$) when all N treatment combinations were considered simultaneously (Figure 3b,d,f). Phosphorus additions weakly increased microbial C- and N-related activity (C enzymes: $F_{1,342} = 3.01$; $p = .084$; N enzymes: $F_{1,291} = 3.51$; $p = .062$), and decreased P-related activity ($F_{1,291} = 3.29$; $p = .070$) in the rooibos tea when all P treatment combinations were considered simultaneously (Figure 3b,d,f). More in-depth exploration showed that the main difference in phosphatase activity was between the P-only and NK + μ treatments (Tukey test: $z = -3.00$; $p = .055$), indicating the potential usefulness of phosphatase activity measured on decomposed plant remains as an indicator of P limitation for microbial decomposition across global grasslands. In contrast, K + μ additions reduced β-glucosidase activity measured on green tea (Figure 2c). Moreover, when all treatment combinations were considered simultaneously, K + μ additions reduced the activity of C- and N-related enzymes on the green tea substrate (C enzymes: $F_{1,346} = 3.60$; $p = .059$; N enzymes: $F_{1,346} = 6.27$; $p = .013$; all K + μ treatment combinations included).

Although nutrient addition generally increased decomposition and biochemical transformation of buried plant remains across our study sites, decomposition parameters and effects sizes in response to treatments varied greatly among and within sites (Figures 1, 2 and 4; Figures S2–S5). For example, the decomposition rate (*k*) increased with increasing microbial enzyme activity measured on the rooibos tea and mean annual precipitation and decreased with mean

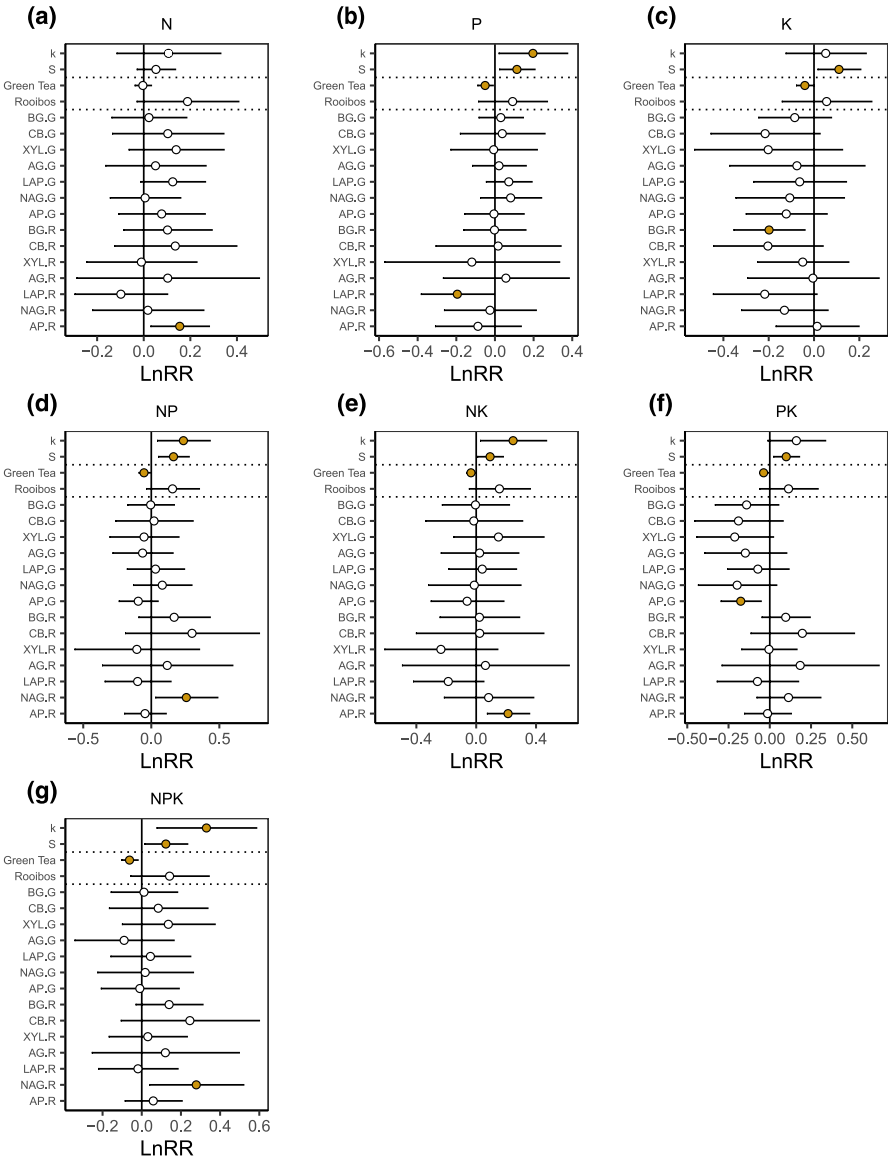

FIGURE 2 Nutrient addition effects on initial decomposition rate (*k*) and stabilization factor (*S*) of plant remains, mass loss of green and rooibos and microbial exoenzymes related to the main biogeochemical cycles (C, N and P) measured on the partially decomposed green tea (G) and rooibos (R). AG, α-glucosidase; AP, acid phosphatase; BG, β-glucosidase; CB, cellobiohydrolase; LAP, leucine aminopeptidase; LnRR, natural logarithm of the response ratio ($variable_{treatment}/variable_{control}$); NAG, *N*-acetyl-glucosaminidase; XYL, xylosidase. Error bars are 95% confidence intervals of the response across experimental sites and treatments. Variables whose error bars do not cross the zero line are shown in orange and are significant at $p < .05$ [Colour figure can be viewed at wileyonlinelibrary.com]

annual temperature (Table S3; Figure 4a–d). The stabilization factor was positively related to microbial phosphatase activity measured on rooibos substrate (Table S3; Figure 4e). Moreover, climatic conditions mediated the effects of nutrients on decomposition. For example, sites with lower mean annual temperatures were associated with more positive effects of N additions on the decomposition rate (*k*; Figure 4b). We also found more positive effects of P additions on *k* at sites receiving higher mean annual rainfall (Figure 4d), while the enhancing effects of fertilization on *S* were conditional to sites with higher mean annual temperatures (Figure 4f).

Finally, our SEM explained 25% of *k* variability and 31% of *S* variability (Figure 5). We found that the consistent positive effects of N and P addition on *k* and *S* were highly dependent on climatic variables and operated 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, N and P fertilization increased these responses most in wetter (*k*) and colder climates (*k* and *S*).

4 | DISCUSSION

Our results demonstrate that the acceleration of initial decomposition rates (*k*) and increased biochemical transformation 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} $year^{-1}$ enhanced decomposition rates across ecosystems (Knorr et al., 2005; Zhang, Luo, Chen, & Ruan, 2018), but are in contrast with a global study in tidal wetlands that showed that *S* was negatively affected by N additions (Mueller et al., 2018). Our results also provide the first empirical evidence that microbial decomposition of aggregate-unprotected plant remains is limited by N and P availability (Hobbie & Vitousek, 2000; Sinsabaugh et al., 1993), but show that other essential nutrients (K + μ) are also relevant drivers of plant matter decomposition at the global scale (Kaspari et al., 2008; Kaspari & Powers, 2016; Keiluweit et al., 2015; Ochoa-Hueso

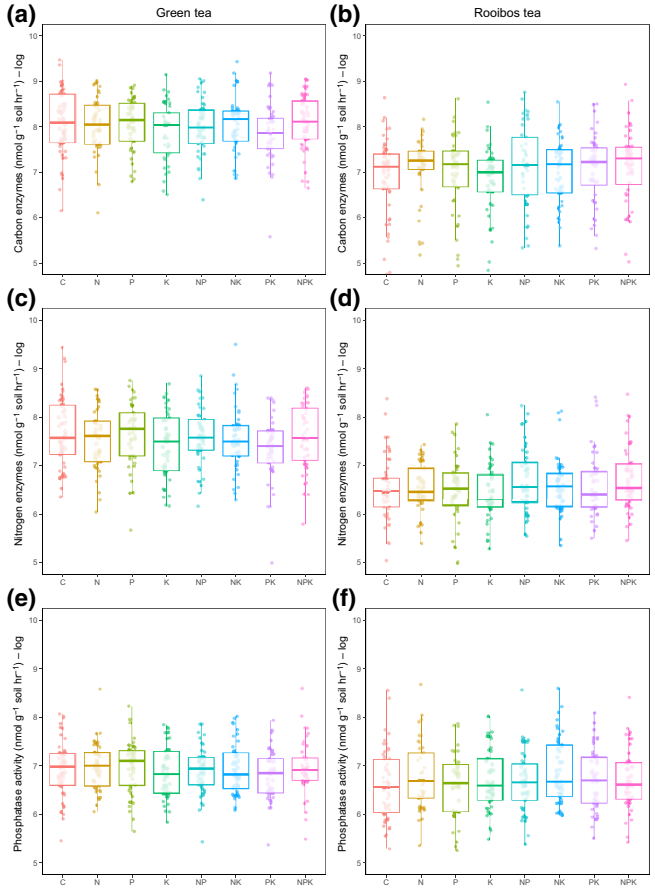

FIGURE 3 Boxplots of effects of nutrient addition on microbial enzyme activity related to the carbon, nitrogen and phosphorus biogeochemical cycles measured on the decomposed plant remains depending on substrate type (green tea [panels a, c and e] vs. rooibos [panels b, d and f]). Median and first and third quartile are shown. C, control; K, potassium + micronutrients; N, nitrogen; P, phosphorus. Substrate types: C enzymes are the sum of four enzyme activities: α-glucosidase + β-glucosidase + cellobiohydrolase + xylosidase. N enzymes are the sum of two enzyme activities: *N*-acetyl-glucosaminidase + leucine aminopeptidase. Detailed results from linear mixed effects models are provided in Table S2. Data points are log-transformed enzyme activities for each plot [Colour figure can be viewed at wileyonlinelibrary.com]

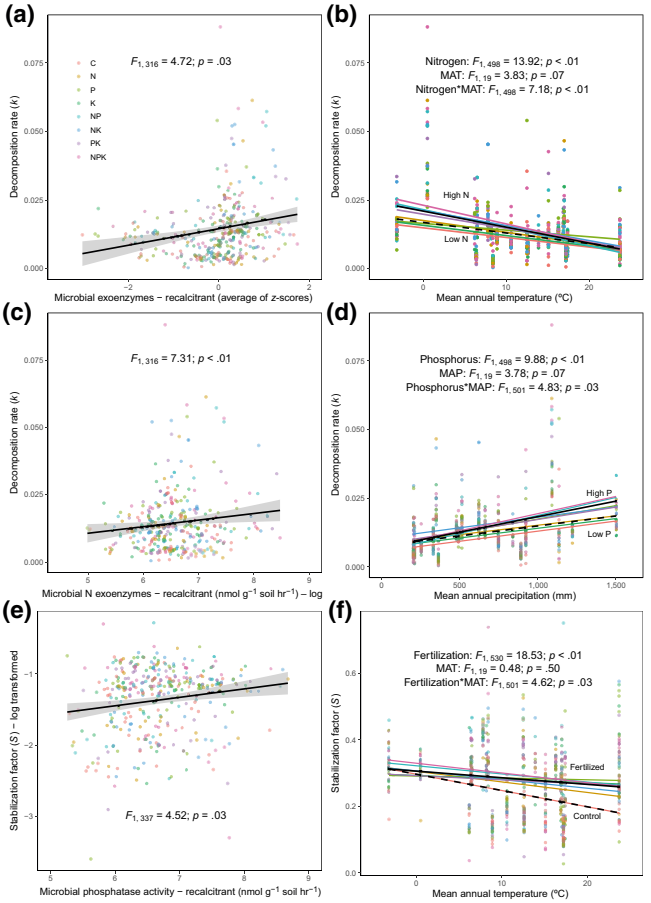

FIGURE 4 Relationships between initial decomposition rate (k) and stabilization factor (S) and their most relevant microbial (a, c, e) and climatic (b, d, f) controllers based on the results of mixed models presented in Table S3. Data points are means for each plot. Solid line in panels (a, c, e) is the fitted linear model across all plots. In panel (b), dashed and solid lines are the fitted linear models under low-N and high-N conditions respectively. In panel (d), dashed and solid lines are the fitted linear models under low-P and high-P conditions respectively. In panel (f), dashed and solid lines are the fitted linear models under control and fertilized conditions respectively. Colour lines in (b, d, f) are based on the legend in panel (a) and are the fitted linear models for each experimental treatment. Enzyme activities are log-transformed [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2019), and thus should not be overlooked. Moreover, our results indicate a limited potential for nutrient management to alter plant residue decomposition in global grasslands due to the moderate magnitude of the effect and its great variability across sites. However, our results are incomplete due to the 90 day duration of our incubations, which serve as a key indicator of early decomposition trajectories but may not reflect cumulative long-term effects of soil nutrient enrichment on decomposition across grasslands.

Despite these general patterns in the response of decomposition to nutrient addition, decomposition of buried plant remains varied widely across our study sites likely due to variations in local climatic conditions and the site-level 'metabolic toolkit' of soil microbial communities to process plant remains. For example, our results of greater biochemical transformation under lower temperatures are concordant with a global study in tidal wetlands (Mueller et al., 2018). Our results also are concordant with the negative relationship between site-level temperature of the wettest quarter and laboratory net N mineralization in global grasslands, but contrast with the positive relationship found between the two when soil incubations were carried out in the field (Risch et al., 2019). Moreover, greater decomposition with increasing rainfall is consistent with a global study using tea bags (Djukic et al., 2018), thus reinforcing the role of climate as a main driver of early-stage litter decomposition across terrestrial ecosystems.

Superimposed to the role of climate and the metabolic toolkit of soil microbes for decomposition, the addition of nutrients significantly altered some relationships of decomposition parameters with

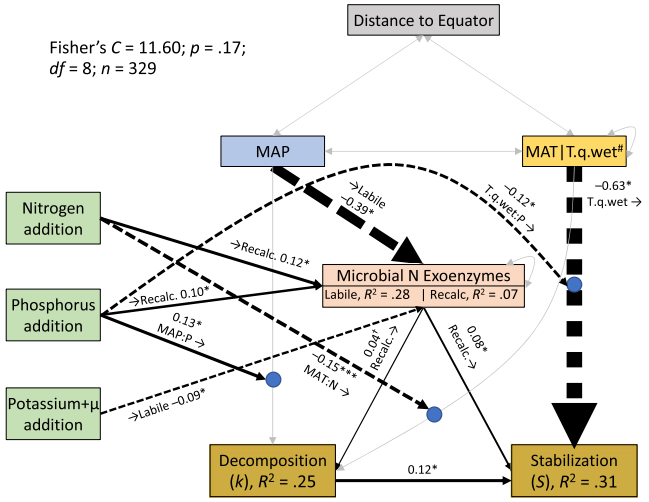

FIGURE 5 Structural equation model. Solid lines = positive associations. Dashed lines = negative association. Line width is proportional to the strength of the association. Bi-directional grey arrows indicate variables with correlated error terms. ***$p < .001$; *$p < .05$; †$p < .1$. Microbial N exoenzymes = microbial enzymes related to the N biogeochemical cycle measured on the decomposed green tea (labile) and rooibos (recalcitrant) substrates. The full a priori conceptual model (i.e. with non-significant paths included) can be found in Figure S14. Significant and non-significant path coefficients as well as coefficients of correlated error terms can be found in Table S4. Arrowheads pointing to blue dots indicate significant interaction terms. MAP, mean annual precipitation; MAT, mean annual temperature; T.q.wet, temperature of the wettest quarter. #MAT in the case of arrows affecting *k* and T.q.wet in the case of arrows affecting *S* [Colour figure can be viewed at wileyonlinelibrary.com]

climatic conditions, including rainfall and soil microbial enzymes. These results further demonstrate widespread co-limitation of decomposition by the availability of water and multiple essential nutrients; factors that are also important for plant productivity and soil C capture in global grasslands (Crowther et al., 2019; Eskelinen & Harrison, 2015). These results indicate the strong coupling between multiple nutrient limitation, soil eutrophication and climatic factors, with likely complex consequences for the global C cycle under future fertilization regimes/nutrient pollution scenarios and warmer and drier climates (Falkowski et al., 2000).

Microbial enzyme activity was consistently higher in the labile substrate, which likely reflects the greater ability of soil microbial communities to quickly colonize and decompose more labile substrates with greater proportion of hydrolysable macromolecules (Chapin, Matson, & Mooney, 2002). Moreover, the downregulation of microbial activity under K + μ additions suggests that the release of some of these enzymes may be associated with the mining of other essential macro- and micro-nutrients from labile organic substrates when these are in short supply. These results show that the metabolic expression of microbial communities differed across the experimental treatments and plant matter substrates, likely due to changes in the composition and abundance of soil bacterial and fungal communities, as described before (Allison, 2012; Leff et al., 2015). These results also suggest that shifts in the composition of plant communities and associated changes in the quality of their dead matter inputs due to eutrophication may further alter the functioning of soil microbial communities (Bjorkman et al., 2018; Bradford et al., 2016).

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

Taken together, our short-term incubations indicate that precipitation and temperature are main drivers of early-stage microbial litter decomposition across terrestrial ecosystems. They also indicate that the microbial decomposition of buried plant remains is weakly but consistently co-limited by the availability of multiple essential macro- and micro-nutrients in grasslands worldwide and will respond interactively to climate variations and soil eutrophication. Adding limiting nutrients to managed grasslands may thus appear as a viable strategy to enhance soil C cycling and perhaps, ultimately, increase soil C sequestration (Prescott, 2010). This may occur via greater biochemical transformation of physically unprotected plant remains that are in close contact with the soil and which may presumably become more recalcitrant humic compounds after the transformation (Conant, Cerri, Osborne, & Paustian, 2017; Prescott, 2010). However, our results also imply that the outcomes of these efforts may be weak and hampered by global warming and the increased frequency of drought events. Nonetheless, this climatic dependency and the known widespread negative consequences of N deposition and adding mineral fertilizers for above- and below-ground grassland biodiversity (Borer, Seabloom, 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 biochemical transformation of plant remains.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Details of authors' contributions can be found in Table S5.

DATA AVAILABILITY STATEMENT

Data used for this study can be accessed through https://figshare.com/articles/Microbial_processing_of_plant_remains_is_co-limited_by_multiple_nutrients_in_global_grasslands/12204380

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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