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Low-intensity management promotes the soil priming effect in European agroecosystems

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Low-intensity management promotes the soil priming effect in European agroecosystems

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Low-intensity management promotes the soil priming effect in European agroecosystems

ABSTRACT

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Agricultural management is critical in shaping soil carbon (C) stocks, pools and fluxes. The soil priming effect (PE) is known as a key component of the global C cycle that reflects alterations in soil organic carbon (SOC) mineralization induced by fresh C inputs. Here, we show that priming can help to predict soil C content across European Long-Term Experiments (LTEs), a result which was maintained at continental and global scales. Results reveal that lower-intensity management significantly enhances PE in soils from European croplands. Conversely, high-intensity management led to lower or even negative PE. Management intensity influences PE directly through alterations in SOC and indirectly by modifying aggregates stability and microbial biomass. Both fertilization and tillage affect PE, with soils under organic fertilization and no-tillage showing higher values of PE. These findings advance our understanding of the long-term impacts of agricultural management on the C cycle at the continental scale.

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INTRODUCTION

Conventional agriculture plays an essential role in shaping food security, but it is also known to have a negative impact on soil organic carbon (SOC) worldwide [1,2]. Agriculture intensification is especially critical in shaping SOC, yet, the mechanisms behind SOC build-up are poorly understood. The soil priming effect (PE) -defined as the change in microbial decomposition of soil organic carbon (SOC) in

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response to fresh carbon (C) inputs (such as litter inputs, or root exudation rich in carbohydrates)- is a crucial component of the global C cycle [3,4]. PE influences the soil's ability to function as a C sink or source. A positive PE can be attributed to an increase in the production of extracellular enzymes by microbes triggered by added substrates that "co-metabolize" soil organic matter. It can also be driven by enhanced microbial activity due to the addition of nutrients like nitrogen (N) and the exploitation of SOC to acquire more limiting nutrients [4–6]. Conversely, the input of fresh C into the soil can reduce the microbial degradation of SOC, a phenomenon referred to as "negative priming" [7]. Strikingly, despite this connection between SOC and PE, the contribution of PE in shaping SOC stocks remains largely undetermined. In the current context of the European Commission's Common Agricultural Policy and the need to advance soil management practices that enhance C sequestration [8], it is essential to consider the connection between PE and SOC in agricultural lands across large environmental gradients.

Agricultural management practices strongly impact SOC turnover and its interactions with the global C cycle, with PE serving as a key mechanism in this process [9,10]. The intensity of the PE is heavily influenced by abiotic factors, including temperature, soil pH, and moisture, as well as biotic factors, such as microbial biomass, diversity, community structure, plant species, etc. [11–13]. Within soil variables, SOC content has been revealed as a critical factor regulating the soil PE at the global scale [9] and, therefore, if significant SOC-PE connection exists across croplands, any soil management practice that ultimately affects the SOC (i.e., tillage intensity or fertilization type) deserves further attention to predict the direction and magnitude of the soil PE. Indeed, numerous studies have demonstrated that less intensive management, particularly through reduced tillage intensity and the application of organic fertilizers, can promote soil C accumulation [14–16], and can potentially enhance positive PE. However, the long-term effects of management intensity on PE remain to be elucidated. Management intensity, as dependent on the level of tillage and fertilization type, exerts a different effect on the biotic and abiotic properties of the soil. Long-term no-tillage often leads to an accumulation of SOC and nutrients in the topsoil layer [15,17]. Further, changing tillage management can deeply alter the chemical [18,19]and microbiological properties [20,21] and result in different PE intensities and direction over the long term. Previous studies have provided valuable insights into PE under different management practices with contrasting results. This inconsistency likely reflects the scarcity of long-term, continental-scale studies across croplands that encompass broad climatic and soil gradients as well as a wide range of management intensities, which limits the representativeness and robustness of current conclusions.

Here, we quantify the contribution of PE to explain SOC aiming to establish a significant connection across croplands, and further evaluate the effects of different management practices on soil PE on a continental scale in eight European agricultural long-term experiments (LTEs). We hypothesized that PE can explain a unique portion of variation in SOC across the EU and further posit that soils subjected to intensive management practices (i.e., standard tillage and mineral fertilization) will have reduced PE (i.e., less SOC mineralization) compared with soils under more sustainable management (i.e., reduced tillage and organic fertilization). Soils with less intensive management (i.e. reduced tillage, application of organic amendments) will accumulate more organic matter, leading to greater microbial biomass and activity [22]. As a result, fresh carbon inputs will stimulate soil microbes, causing additional SOC mineralization - positive PE-, which will be more pronounced in these soils than in those with more intensive management. To test these hypotheses, we measured PE in soils from agricultural ecosystems with long-term -at least eight years- of different tillage and fertilization managements across seven European countries, covering three major climate types (semi-arid, temperate, and cold). We further categorized these soils into four management intensity levels, which were dependent on the level of tillage and fertilization type (mineral vs organic), and modelled the abiotic and biotic drivers of the soil PE across the studied LTEs. Finally, we cross-validated the results associated with our first goal using two independent EU and global databases aiming to establish a strong and significant connection between PE and SOC.

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RESULTS

Priming effects explain a unique portion of soil carbon

We first investigated the capacity of PE to explain SOC in a LTE agricultural network across the EU, accounting for multiple environmental factors such as climate, microbial biomass, soil properties and spatial distribution, using two independent approaches: a multi-regression linear approach and a variation partitioning approach. PE was selected as an important predictor of SOC within the top five more significant models (Figure 1a). Moreover, PE explained a unique portion of the variation of SOC, not accounted for other environmental factors (Figure 1b). We further cross-validated our results using two independent continental-EU (The LUCAS survey) (Figure S1; LUCAS)[10] and global scale (Fig. S1A; Global)[9] databases including agricultural and natural ecosystems with a greater number of locations, and supporting stronger statistical power. In both cases, PE played a critical role as a predictor of SOC in the top best models, and in explaining a unique portion of the variation of SOC across large environmental gradients (Figure S1).

Higher-intensity management reduces the soil PE

Management intensity significantly impacted the strength and direction of the PE ($p < 0.01$; Figure 2a). Higher management intensities (H) resulted in a significantly weaker PE, with mean values of 3.18 ± 1.36 μg C g^{-1} soil . In contrast, lower management intensities (L and ML) exhibited a stronger PE, with mean values of 11.43 ± 5.10 μg C g^{-1} soil for L and 10.63 ± 2.57 μg C g^{-1} soil. In accordance, and despite the spatial heterogeneity of soil samples leading to considerable data variability, higher management intensities (MH and H; -24.32 to 17.86 μg C g^{-1} soil) typically exhibited lower or even negative PE (Figure 2a), whereas in most cases, lower management intensities were associated with positive PE (L and ML; -22.82 to 47.11 μg C g^{-1} soil). When separating factors -fertilization type and tillage level-analysis revealed that soils subjected to organic fertilization showed higher PE than those subjected to mineral fertilization (Figure 2b). Similarly, non-tilled soils had greater PE than soils with reduced or standard tillage (Figure 2c). PEs were, on average, higher in field trials conducted in Slovenia (ULFB), Austria (BOKU), and Sweden (SLU) than in those carried out in other regions, such as Spain (INIA), Italy (CREA), etc. (Figure S2).

Further, microbial biomass (EL-FAMES) was measured after incubation and results were expressed as the difference between glucose-amended soil samples and non-amended samples (Table S1). This allowed us to explore if glucose induced enhancement of microbial biomass and mechanistically tested if this response could explain partially PE patterns. Overall, glucose induced a enhancement of microbial biomass, in particular in the case of bacteria (Table S1). Fertilization type showed minor effects on the stimulation of microbial biomass upon glucose addition. In contrast, PERMANOVA revealed significant effects of tillage in bacterial biomass response after glucose, with higher values in standard tilled soils (ST) compared to non-tilled (NT) and reduced-tilled (RT) ones. Further, management intensity revealed a consistent trend whereby glucose addition stimulated microbial biomass, especially in soils subjected to high disturbance intensity.

Soil PE predictors

Random Forest showed that C/P ratio, SOC, TN, and management intensity were significant predictors of the soil PE (Figure 3a; Table S3). In concordance, regression analyses showed that PE was significantly and positively correlated with C/P ratio, SOC and TN (Figure 3). The SEM results showed that management intensity, SOC, and β-glucosidase activity directly influenced soil PE (Figure 3b; Table S3). Additionally, management intensity indirectly affected PE by altering soil MWD, fungal biomass, and bacterial biomass, which in turn contributed to PE variations. According to our SEM, SOC and β-

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glucosidase activity had positive and negative direct effects, respectively, on the soil PE. Soil MWD, fungal biomass, and bacterial biomass exerted indirect effects, all regulated by management intensity (Figure 3c). Consistently, linear regression analysis further confirmed that PE was significantly positively correlated with SOC and MWD, indicating that increases in these factors significantly enhanced PE (Figure 4). In addition, we tested the associations between PE and the relative abundance of different microbial groups as retrieved by 16S rRNA and 18S rRNA gene sequencing (Fig. S3). Among bacterial orders, we found positive and significant ($P < 0.05$) associations with Acidimicrobiales, Tissierellales, Methanosarcinales, Gp18, Limnochordales, Gp25, Chitinispirillales, and negative associations with Nitrosomonadales and Streptomycetales.

DISCUSSION

Priming effect explains a unique portion of soil C content and is shaped by agricultural management

Our work provides evidence that PE can explain a unique portion of variation in the large-scale distribution of soil C across croplands, identifying a significant connection between these two important aspects of C cycling. Our results were cross-validated in a continental-scale survey across the EU, and in a global survey across natural environments; both databases available online [9,10]. After establishing this connection, we further evaluate the role of agricultural intensification in shaping PEs, and found that agricultural intensity influences PE directly through alterations in SOC and indirectly via changes in aggregates stability and microbial biomass. These results reveal a strong association between PE and soil C content. This knowledge is critical to better predict the fate of SOC in croplands under global change.

In our study, positive PE was observed in 66.3% of soil samples. This indicates that, in most instances, glucose addition consistently enhanced the mineralization of SOC [5]. Previous studies also reported positive PE following different agricultural management practices [23,24]. Positive PEs are generally attributed to microbial growth and the subsequent increase in enzyme production, which accelerates the mineralization of SOM in order to mine nutrients [25–27]. However, as shown in other studies [28], the soil PE is tremendously variable, particularly at the continental scale [10]. Further, the soil PE induced by lower-intensity management was significantly greater than that induced by higher management intensity. Under low-intensity management (i.e. including reduced tillage and organic fertilization), the biomass and activity of soil microbial communities were relatively high (Figure S4). The addition of easily decomposable low-molecular-weight substrates, such as glucose, can rapidly activate dormant microorganisms [29,30] or being decomposed by the existing microbial biomass. Moreover, soils subjected to low-intensity management tend to be more carbon-saturated [31] and therefore the added glucose is likely to be readily available to soil microorganisms. Indeed, glucose-mineralization was greater in soils under low management intensity compared to high-intensity ones (Table S2), despite the fact that microbial biomass did not increase following glucose addition in the low-intensity managed soils. Therefore, PE observed in lower-intensity managed soils mainly reflects an accelerated turnover of the existing microbial biomass [4] or competitive processes for nutrient acquisition, rather than on SOC mineralization. In contrast, soils under higher-intensity management, which exhibited lower SOM, microbial biomass, and nutrient contents, showed a trend toward increased microbial biomass after glucose addition, while glucose mineralization was lower than in soils under lower-intensity management. Therefore, these results indicates that mineralizaction of SOC and positive PE could occur through the mining of nutrients [32,33] that are less available or abundant in those soils compared to soils under low-intensity management. Overall, results highlight that the legacy of management in croplands can determine the mechanism for which PE occur in European croplands. Nevertheless, as these results are derived from an incubation assay, we acknowledge that continuous inputs of fresh organic matter under field conditions could render different effects on PE and SOC content.

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Although positive priming effects were more frequently observed across the entire sample group, 22.2% of samples from low-intensity agricultural management exhibited a negative PE, while this proportion increased to 44.7% in soils subjected to high-intensity use. This result indicates that high-intensity management led to a greater reduction in soil C mineralization, resulting in a higher proportion of soils exhibiting a negative PE. The low levels of organic carbon and nutrients under high intensivity management (Figure S4) probably limit microbial heterotrophic respiration since PE requires the utilization of pre-existing organic matter. Moreover, negative PE can occur due to preferential substrate utilization, whereby microorganisms shift from decomposing stable SOC to mineralizing the added fresh carbon [5,7,10]. This phenomenon is often associated with limited C availability, particularly in soils dominated by recalcitrant C [10]. The higher proportion of negative PE under high management intensity reflects lower C availability in soils subjected to long-term standard tillage and mineral fertilization. In line with this, we observed a stronger stimulation of microbial biomass after glucose addition in these high-intensity soils, especially under standard tillage (Table S1), which were also those showing negative PE. Long-term intensive practices reduced SOM and microbial biomass (Figure S4), but increased microbial C limitation and responsiveness to labile C inputs, consistent with preferential substrate use [32,34].

We also analyzed the effects of isolated practices (tillage and fertilization). Both agricultural management practices (tillage and fertilization) had a significant impact on the PE, with no significant interaction observed between them. Our results showed that organic fertilization (OG) induced a stronger PE, which is consistent with previous findings. The application of organic materials can stimulate microbial activity and extracellular enzyme synthesis to acquire essential nutrients, particularly N, thereby accelerating SOC mineralization and enhancing positive PE [35,36]. Furthermore, our study revealed that NT resulted in a higher PE, which appears to be inconsistent with previous studies reporting either no significant effect or a reduced PE under NT conditions [37,38]. This may be attributed to the higher microbial biomass and enzyme activity in no-tillage soils, which help to enhance the soil PE. The high enzyme activity and nutrient availability in no-tillage soils provide resources for microbes, allowing them to simultaneously degrade both easily (i.e. added glucose, Table S2) and more stable SOM sources, thereby enhancing the soil PE.

Drivers of the soil PE

Agricultural management practices indirectly regulate PE through the modulation of SOC, while also influencing PE via alterations in MWD and microbial community. Microbial communities (bacterial and fungal biomass) were more abundant under lower-intensity management practices than in high-intensively managed soils. These microorganisms might be more efficient in decomposing external carbon sources (such as glucose), thereby accelerating the mineralization of SOC and ultimately leading to higher PE. SEM revealed a positive relationship between total nitrogen (TN) and PE that contradicts the predictions of the classical Nutrient Mining Hypothesis [3,39]. Indeed, Random Forest analysis identified TN as a key predictor of PE, and regression analysis further confirmed a significant positive correlation between N content and PE (Figure 3b). This finding indicates that, in high-TN soils as those with less intensive management, microbial communities experience less N limitation and are "ready" to more efficiently utilize external C inputs (e.g., glucose, Table S2), thereby promoting the co-metabolic decomposition of SOC and PE once microbial community has evolved and require more nutrients [40,41]. Further, SEM highlighted that β-glucosidase plays a vital role in PE patterns. This enzyme is responsible for breaking down complex organic matter into simple sugars, thus providing a C source for diverse microbial communities [42]. By accelerating SOC decomposition, β-glucosidase enhances CO_2 release [43]. In soils with lower β-glucosidase activity, the decomposition efficiency of soil C compounds is reduced, making glucose addition more likely to trigger a higher PE through the stimulation of soil microbial communities.

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When C limitation in soil is alleviated through addition of glucose, other nutrients become the secondary limiting factor for microbes [44,45]. P mining theory suggests that the input of fresh C promotes microbial growth and increases their demand for P, intensifying nutrient limitations. In these circumstances, soil microbes decompose SOM to acquire P, simultaneously promoting PE [46]. The positive relationship between the soil C-to-P ratio and the soil PE suggests that mining of SOM (though positive PE) can act as a mechanism for alleviating P limitation in European agroecosystems. Moreover, regression analysis revealed a significant positive association between MWD and PE, suggesting that larger aggregates can create favorable conditions for microbial activity and further enhance the mineralization of SOC [47,48]. Further, the significant association between urease activity and PE highlights the role of this enzyme in satisfying N microbial demand during SOM decomposition, as mining of the SOM has been shown to be a critical mechanism explaining PE [49,50]. These findings further underscore the pivotal role of enzyme activities in shaping both the direction and intensity of the PE. Our study further explored the associations between the relative abundance of microbial taxa and PE. The negative relationship observed between Nitrosomonadales and PE reflects the role of this group in nitrification, whereby increased availability of inorganic nitrogen reduces the need for heterotrophic microorganisms to mine nitrogen from SOM. In contrast, the positive association between Acidimicrobiales abundance and PE suggests that these oligotrophic Actinobacteria promote SOM decomposition through enzyme-mediated mining of complex organic substrates, a process that is further stimulated by the addition of labile carbon. Indeed, several studies have highlighted the role of Actinobacterial groups to degrade fresh organic matter but also necromass [51,52].

Limitations of this study

Although the present study represents a step forward in the understanding of the effect of agricultural management intensity on PE across Europe, it has several limitations that we would like to highlight. The sieving process used in this study can alter soil aggregate structure, potentially affecting CO_2 release, microbial activity, and nutrient cycling [53]. While sieving is a necessary step to ensure consistency in incubation experiments, it may disrupt natural soil aggregation and influence microbial accessibility to organic matter. PE can be influenced by both the quantity and quality of soil organic carbon (SOC) associated with different aggregate size fractions; therefore, the disruption of aggregates caused by sieving could potentially modify PE responses. However, evidence linking aggregate disruption to changes in PE remains inconclusive and appears to vary with soil type and management context. For example, You et al. [54] reported that reducing aggregate size did not alter PE in a Mollisol, suggesting that PE was primarily driven by labile carbon inputs or fresh litter rather than by SOC protected within aggregates. In contrast, Tian et al. [55] observed that aggregate crushing did affect PE in a Haplic Luvisol, although the pattern depended on the glucose dose applied. Moreover, the controlled incubation conditions (e.g., temperature and moisture, a single type of added C molecule, etc.) did not fully mirror those prevailing in the field. Therefore, our results should be interpreted as indicative of potential priming patterns rather than exact *in situ* responses. Nevertheless, such controlled experiments provide valuable insights for evaluating assumptions in microbially-explicit soil biogeochemical models and for understanding how the legacy of long-term soil management may shape microbial processes and edaphic factors driving PE at continental scale.

Our study quantifiedthe contribution of PE in explaining soil C content in croplands across large environmental gradients, and further identified the role of agricultural intensification in shaping PE, with consequence for C cycling under global change. Thus, we comprehensively explored the impact of management intensity on soil PE across European LTEs, revealing the complex mechanisms by which different management practices regulate soil health and C cycling. Overall, results highlight that the

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legacy of management in croplands can determine the mechanism for which PE occur in European croplands. In the long term, low-intensity management improves soil health by enhancing microbial biomass, enzyme activity, and TN while increasing SOC. Interestingly, these benefits of sustainable agricultural practices are accompanied by enhanced soil C turnover through increased PE in European agroecosystems. However, this does not diminish the crucial role of reduced tillage and organic fertilization in enhancing SOC content in European agroecosystems.

METHODS

Experimental design and soil sampling

The selected LTEs, within the EJP Soil MINOTAUR project, encompass a large gradient of edapho-climatic conditions and are located across seven countries, namely Spain, France, Italy, Slovenia, Austria, the Netherlands, and Sweden (Dataset 1). These LTEs represent a long-term network of sites, with some of them starting in 1974 (i.e., Sweden) and the newer ones in 2012 (France) (Dataset 1). All experiments assess the effects of minimizing soil disturbance by comparing standard tillage (ST) vs reduced (RT) or no-tillage (NT) and/or implementing distinct fertilization approaches: mineral (MN) vs organic (OG). Three plots per treatment and site were established. Soil organic carbon and nitrogen content differ markedly among the LTEs, with Sweden (SLU) and Slovenia (ULBF) exhibiting the highest levels, while Spain (INIA) the lowest. Soil acidity also varies, with Austria (BOKU), Italy (CREA), and the Netherlands (WUR) having basic soils (pH 7-8) and higher nutrient availability, compared to the more acidic soils (pH 5-6) in France, Sweden and Austria. Furthermore, soil texture shows notable differences, with Sweden and Slovenia having higher clay content (30-40%) than the other sites (13-20%). In terms of climate, the Swedish site represent the coldest end of the spectrum with an average annual temperature around 6°C. In contrast Spanish (INIA) and Italian (CREA) sites, located in the Mediterranean regions, experience warmer temperatures with annual averages of 13°C and maximum means around 24°C. The LTE sites also vary in precipitation, with ULBF, AGS, and CREA receiving over 1000 mm annually, INIA the lowest at around 400 mm, and the rest falling between 500 and 800 mm.

Based on the level of tillage and type of fertilization, LTE treatments were categorized into four management intensity levels: L (low intensity), ML (middle low intensity), MH (middle high intensity), and H (high intensity). These levels were determined according to the combination of tillage intensity and fertilization types. L includes organic fertilization with reduced tillage or no-tillage (OG-RT and OG-NT, respectively); ML includes organic fertilization with standard tillage and soil subjected to mineral fertilization and no-tillage (OG-ST and MN-ST, respectively); MH includes soils with mineral fertilization and reduced tillage (MN-RT); and H includes soils with mineral fertilization and standard tillage (MN-ST).

Soil samples (0-10 cm) were collected between late summer and early autumn 2022. In each plot, five subsamples were collected in the center of the plot and in the four cardinal points at 2 m distance from the center (following the LUCAS methodology [56]), and subsequently mixed into a composite sample per plot. After field sampling, the soil was sieved (2 mm). Each sample was portioned in three parts: one was air-dried for chemical analyses, one was stored at 4ºC for biochemical analyses and the last was stored at -20ºC for microbial biomass estimations. For aggregate stability measurements, an intact soil block (~15 × 20 × 10 cm) was collected and partially air-dried on trays. The block was then gently disaggregated by hand, passed through a 3–5 mm sieve, dried at 40 °C for 24 h, and stored at 4 °C until analysis.

Soil analyses

For all soil samples, we measured their physical and chemical properties, including texture (sand, clay, silt contents), pH, SOC, total nitrogen (TN), total phosphorus (P), bulk density (BD) and aggregate stability (MWD). Specifically, total carbon (TC) and nitrogen (TN) were determined in aliquots of finely ground soil (<100 μm) using an elemental analyzer (CNS 628, Carlo Erba Instruments, Italy); total organic C (TOC) was also measured after $CaCO_3$ removal with 20% HCl, using the 'capsule method' of Brodie et

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al. [57]. Soil pH was determined in a suspension in distilled water and in 1 M KCl (1:2.5 soil:solution ratio) as described by Guitián-Ojea and Carballas-Fernández (Guitián Ojea et al., 1976). Particle size distribution was determined using a Robinson pipette with Calgon as dispersant, after oxidation of the organic matter with H_2O_2 (Guitián Ojea et al., 1976), and the textural class was assigned according to the USDA soil texture classification [58]. The pseudo-total content of P was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, model Vista-PRO), after acid digestion with "aqua regia" (HNO_3 +HCl) in a microwave oven (MILESTONE, model ETHOS, Italy).

Aggregate stability, expressed by the mean weight diameter (MWD, mm), was determined using the method of Le Bissonnais [59] and ISO [60], which considers three soil aggregate breakdown mechanisms: slaking (fast wetting), microcracking (slow wetting), and mechanical breakdown (stirring after pre-wetting). The final MWD reported here represents the average of the three method-specific MWDs, with higher values indicating greater aggregate stability [59], BD ($g \cdot cm^{-3}$) was measured using the cylinder method [61]: after sampling a volume of undisturbed soil in the field using a cylinder of known volume, in the laboratory, after drying at 105°C, the soil mass is measured and the BD is calculated.

The activities of acid and alkaline phosphomonoesterase, and β-glucosidase were determined at their optimum pH by incubating the soils with a substrate containing a p-nitrophenyl moiety, followed by measuring the amount of p-nitrophenol released using spectrophotometry at 400 nm. The optimal pH for each enzyme activity was initially determined by measuring the activity at different pH levels in several soil samples from each LTE and treatment. The method described by Trasar-Cepeda et al. [62] was then applied to ensure that the pH of the reaction mixture was maintained. Acid phosphomonoesterase activity was determined at pH 6.50, except for the ACO samples, which were measured at pH 6.0. Alkaline phosphomonoesterase activity was measured at pH 10.5, except for the ACO, SLU and some of the AGS samples which were measured at pH 10.0. Both acid and alkaline phosphomonoesterase activities were measured following the method of Tabatabai and Bremner [63], using 16 mM *p*-nitrophenyl phosphate as the substrate. The samples were incubated with Modified Universal Buffer for 30 minutes, maintaining the reaction mixture at the specified pH. After incubation, 0.5 M $CaCl_2$ was added, and the released *p*-nitrophenol was extracted with 0.5 M NaOH. ß-glucosidase activity was determined at pH 6.0, as described for phosphomonoesterase activity, except that the substrate was 25 mM *p*-nitrophenyl-ß-D-glucopyranoside, the incubation time was 1 h, and the released p-nitrophenol was extracted with 0.1 M (Tris(hydroxymethyl)aminomethane)-NaOH (THAM-NaOH), pH 12 [64]. The activities of each of the above enzymes were quantified by reference to calibration curves corresponding to *p*-nitrophenol standards. Since p-nitrophenol can be retained by the soil, tests were conducted for all soil samples to ensure that retention did not occur. In cases where retention was observed, the standard curves were performed by incubating the p-nitrophenol standards with each soil under the same conditions as for the samples [65,66]. The activities of these three enzymes are expressed as µmol *p*-nitrophenol released g^{-1} h^{-1}. Urease activity was determined following the method described by Nannipieri et al. [67]. In brief, the soil samples were incubated for 1.5 h, at pH 7.0 with 1065.6 mM urea as the substrate. The pH was maintained by using phosphate buffer at pH at pH 7.0, except for SLU, ACO and some AGS samples, for which activity was determined using phosphate buffer at pH 8.0 (necessary to have the reaction mixture at pH 7.0). The NH_4^+ released during the incubation was measured by using an ammonia electrode [68], and the activity is expresses as µmol NH_3 g^{-1} h^{-1}. In all cases, the reported values are the average of duplicate analytical determinations on triplicate samples for each treatment and are expressed on an oven-dried soil basis, 105 °C.

The bacterial and fungal biomass in each soil sample was quantified through the analysis of ester-linked fatty acid methyl esters (EL-FAMEs), referred to as FAMEs. The procedure followed the method described by Schutter and Dick [69]. Briefly, 3 g of soil were treated with appropriate reagents for FAMEs methylation, neutralization, and extraction. The resulting FAMEs were analyzed using an 8860 GC System gas chromatograph (Agilent Technologies, Santa Clara, USA) fitted with a 30-meter DB-

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FastFAME capillary column (Agilent Technologies), as described by Vera et al. [70]. The fatty acids i15:0, a15:0, i16:0, i17:0, 16:1ω7, cy17:0, cy19:0, 10Me16:0, and 10Me18:0 were used as markers for bacterial biomass [71,72], while 18:2ω6,9t and 18:2ω6,9c were used as markers for fungal biomass [73,74].

Total DNA from soil was extracted from 250 mg of fresh soil using the DNeasy PowerSoil Pro Kit (QIAGEN), following the manufacturer's instructions. The prokaryotic (bacterial and archaeal) community was characterized by metabarcoding using the primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-ACGGACTACNVGGGTWTCTAAT-3′). The eukaryotic community was also characterized by metabarcoding using the primers Euk1391F (5'-GTACACACCGCCCGTC-3') and EukBr (5`TGATCCTTCTGCAGGTTCACCTAC-3') [75,76]. Gene amplification, library preparation and sequencing were conducted at the Genomics and Microbiome Core Facility (GMCF) at Rush University (Chicago, USA) according to in-house protocols. Bioinformatic processing of raw sequencing data, including quality filtering, trimming, ASV inference, and chimera removal, was conducted using DADA2 (v. 1.28.0)[76]. Taxonomic assignment of ASVs was performed using Mothur (v. 1.47.0)[75] with the SILVA Small Subunit rRNA database (release 138.2) as the reference database.

Experiment incubation

Soil priming was assessed through a controlled laboratory incubation following the general framework proposed by Bastida et al [9]. Microcosms were established using 1 g of soil placed into 20-mL airtight glass vials, with moisture adjusted to 50% of the soil water-holding capacity. Samples were incubated in darkness at 25 °C for 15 days prior to substrate addition to allow microbial activity to stabilize under standardized temperature and moisture conditions. After this equilibration phase, vials were briefly opened to re-establish ambient headspace composition. Substrate treatments were then imposed by adding a solution of uniformly labelled ^{13}C-glucose (99 atom% U-^{13}C; Cambridge Isotope Laboratories) dissolved in sterile deionized water at a rate of 75 µg glucose-C g^{-1} soil. This application rate was selected based on the comparatively low microbial biomass in these soils relative to values reported by Siles et al. [10] and on previous evidence indicating that only a small fraction (approximately 10%) of added glucose undergoes mineralization. Control microcosms received the same volume of sterile deionized water without glucose addition. A total of 184 microcosms (92 soil samples per group) were incubated in the dark at 25°C for 32 days. This incubation duration allows quantification of the real priming effect rather than transient apparent responses, as discussed by Blagodatskaya and Kuzyakov[40]. At the end of the incubation period, 4 mL of headspace gas were withdrawn from each vial and transferred into pre-evacuated 12-mL Exetainer glass vials (Labco Ltd, High Wycombe, UK) for subsequent determination of CO_2 concentration and isotopic composition. The $\delta^{13}C$ signature of CO_2 was analysed using a ThermoScientific Gas BenchPreCon trace gas system coupled to a Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, Germany) at the UC Davis Stable Isotope Facility, following the analytical procedures described by Bastida et al.[77]. Isotopic data were expressed relative to the international Vienna Pee Dee Belemnite (V-PDB) reference standard, according to Moreno et al.[78]. Partitioning of total CO_2 into glucose-derived and native soil organic carbon (SOC) sources was performed using the measured $^{13}C/^{12}C$ isotopic ratios. Basal respiration was estimated from CO_2 production in control soils receiving only water. The PE was defined as the change in CO_2 derived from SOC mineralization after glucose addition, compared to the control soil with only water added. Glucose-induced priming was subsequently calculated using the equation described below.

$$PE = C_{treatment} - C_{CK} \quad (1)$$

where $C_{treatment}$ is the SOC-derived CO_2 of soil with glucose addition and C_{CK} is the basal CO_2 efflux of soil without glucose addition. The PE was expressed as µg C g^{-1} soil.

Further, microbial biomass as estimated by EL-FAMES (Schutter and Dick [69] was measured after incubation and results were expressed as the difference between glucose-amended soil samples and non-

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amended samples (Table S1). This was used as a proxy to obtain mechanistic information of the changes in microbial biomass during our priming approach.

Statistical analyses

Quantifying the contribution of PE in explaining SOC

Multi-model inference analyses

We evaluated the relative importance of the different covariates (e.g., priming, pH) for explaining soil organic carbon (SOC) using multimodel inference implemented with the *MuMIn* package in R. This approach fits all possible combinations of predictors in a multiple regression framework and computes the Bayesian Information Criterion (BIC) for each candidate model, which is then used to rank models according to the principle of maximum parsimony. BIC quantifies the trade-off between explanatory power and model complexity by penalizing models with unnecessary parameters. The model with the lowest BIC is considered the best, and all other models are ranked based on their BIC deviation. Typically, models with a BIC difference < 2 are regarded as having comparable support; here, to apply a more conservative threshold, we focused on models with $\Delta BIC < 4$ of the best model. Identifying which variables consistently appear in this set of top-ranked models provides insight into their importance. This procedure complements variance partitioning because it is less dependent on the absolute amount of variance explained and more sensitive to whether a predictor uniquely accounts for any component of that variance. Consequently, a variable that explains even a small but unique portion of the variance is typically retained in the best-performing models. More details about this method can been found from Sáez-Sandino et al. [79].

Multiple regression models

We used multiple regression models to evaluate the relative importance of individual predictors including aridity, priming, bacterial biomass, fungal biomass, land use, latitude, pH, PSEA, TSEA, and mean annual temperature (MAT) in explaining variation in soil organic carbon (SOC). Prior to analysis, we calculated variance inflation factors (VIFs) to assess collinearity among predictors and excluded variables exhibiting high multicollinearity (VIF > 10). All predictors and the response variable were standardized using z-scores to allow parameter estimates to be interpreted on a comparable scale. We used the "*relaimpo*" package in R to estimate standardized coefficients and quantify the contribution of each predictor, expressing their relative importance as the percentage of variance explained.

Quantifying the influence of management intensity in shaping PE

The effects of management intensity, type of fertilization and type of tillage on native SOC decomposition and PE were analyzed using ANOVA (analysis of variance). When ANOVA resulted in significant differences, the post-hoc least significant difference (LSD) test was applied to pairwise compare treatments. Normality and heteroscedasticity of data were confirmed by the Kolmogorov–Smirnov and Levene tests, respectively. We further applied simple linear regression analysis to evaluate the relationship between PE and soil properties (SOC, TN, P, C/P, urease activity and MWD). These analyses were conducted using the SPSS software (version 24.0). Before conducting structural equation modeling (SEM) analysis, we used random forest methodology to assess the relative importance of predictors of PE, including management intensity as a categorical variable, soil physicochemical properties (sand, clay, silt, pH, SOC, TN, P, C/N, C/P, C/N/P, MWD and BD), microbial community (fatty acids content as a proxy of bacteria and fungi biomass), and enzyme activities (β-glucosidase, urease, acid phosphatase, and alkaline phosphatase). By constructing bagged tree ensembles and incorporating a random subset of features for each tree ($n_{tree} = 1000$), the random forest analysis effectively could alleviate multicollinearity

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problems in multivariate analyses, while simultaneously enhancing the model reliability and accuracy [80]. The importance (increase in mean square error percentage) and significance of each predictor was computed for each tree and averaged over the forest using the rfPermute package ver. 4.4.1 [81] of R. Significant factors of the PEs were defined as those with $P < 0.05$. Subsequently, we performed SEM analysis to model the direct and indirect pathways through which environmental and biotic variables influence PE based on an *a priori* conceptual model (Figure S5). The hypothesized causal relationships were based on existing knowledge of how biotic and abiotic soil properties impact PE. The initial model was refined by sequentially removing non-significant paths, resulting in the final model that best fits the data. To verify the robustness of the relationship between key ecosystem factors and PE, we employed piecewise SEM to account for the random effects of sampling sites, and assessed the "marginal" and "conditional" contributions of environmental predictors. Fisher's C test was used to evaluate the model's goodness-of-fit, and the model was iteratively refined based on path significance ($P < 0.05$). The SEM was implemented using the "piecewiseSEM" ver. 4.4.1 package [82] in R version 4.4.1 (2024-06-14 UCRT). For transparency, we report standardized path coefficients and overall goodness-of-fit statistics in the main text/figures, and provide R^2 values (explained variance from the component models) for each endogenous response variable in Figure 3 caption. The effects of management intensity, fertilization type, and tillage system on soil microbial biomass (EL-FAMEs) after incubation were assessed by calculating the difference in EL-FAME content between glucose-amended soils and their corresponding non-amended controls. In addition, effect sizes were quantified using eta-squared (η^2) from one-way ANOVA models for each response variable and experimental factor, to evaluate the magnitude of treatment effects irrespective of statistical significance. Finally, Pearson correlation coefficients (r) between PE and relative abundances of bacterial (16S rRNA gene) and eukaryotic (18S rRNA gene) taxa at the phylum and order levels across samples were calculated.

DATA AVAILABILITY

The data used in this study have been deposited in figshare at 10.6084/m9.figshare.31429163 and 10.6084/m9.figshare.31429322.

CODE AVAILABILITY

The code used in this study has been deposited in figshare at 10.6084/m9.figshare.31568962.

REFERENCES

1. Lal, R. Digging deeper: a holistic perspective of factors affecting soil organic carbon sequestration in agroecosystems. *Global Change Biol.* **24**, 3285–3301 (2018).

2. van Rijssel, S. Q. *et al.* Conventional and organic farms with more intensive management have lower soil functionality. *Science* **388**, 410–415 (2025).

ARTICLE IN PRESS

3. Kuzyakov, Y. Priming effects: interactions between living and dead organic matter. *Soil Biology and Biochemistry* **42**, 1363–1371 (2010).
4. Kuzyakov, Y., Friedel, J. & Stahr, K. Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* **32**, 1485–1498 (2000).
5. Blagodatskaya, E. V., Blagodatsky, S. A., Anderson, T.-H. & Kuzyakov, Y. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied Soil Ecology* **37**, 95–105 (2007).
6. Liu, X.-J. A. *et al.* Labile carbon input determines the direction and magnitude of the priming effect. *Applied Soil Ecology* **109**, 7–13 (2017).
7. Guenet, B., Leloup, J., Raynaud, X., Bardoux, G. & Abbadie, L. Negative priming effect on mineralization in a soil free of vegetation for 80 years. *European Journal of Soil Science* **61**, 384–391 (2010).
8. Pe'er, G. *et al.* A greener path for the EU Common Agricultural Policy. *Science* **365**, 449–451 (2019).
9. Bastida, F. *et al.* Global ecological predictors of the soil priming effect. *Nature Communications* **10**, 3481 (2019).
10. Siles, J. A. *et al.* Priming effects in soils across Europe. *Global Change Biology* **28**, 2146–2157 (2022).
11. Blagodatskaya, E. & Kuzyakov, Y. Priming effects in relation to soil conditions–mechanisms. *Encyclopedia of Agrophysics. Dordrecht: Springer* 657–67 (2011).
12. Huo, C., Luo, Y. & Cheng, W. Rhizosphere priming effect: a meta-analysis. *Soil Biology and Biochemistry* **111**, 78–84 (2017).
13. Wang, C.-Q. & Kuzyakov, Y. Soil organic matter priming: the pH effects. *Global Change Biol.* **30**, e17349 (2024).
14. Li, J. *et al.* Soil labile organic carbon fractions and soil organic carbon stocks as affected by long-term organic and mineral fertilization regimes in the North China Plain. *Soil and Tillage Research* **175**, 281–290 (2018).
15. Liu, E. *et al.* Long-term effects of no-tillage management practice on soil organic carbon and its fractions in the northern China. *Geoderma* **213**, 379–384 (2014).

ARTICLE IN PRESS

ARTICLE IN PRESS

16. Romanenkov, V. *et al.* Soil organic carbon dynamics in long-term experiments with mineral and organic fertilizers in Russia. *Geoderma Regional* **17**, e00221 (2019).
17. Thomas, G. A., Dalal, R. C. & Standley, J. No-till effects on organic matter, pH, cation exchange capacity and nutrient distribution in a Luvisol in the semi-arid subtropics. *Soil and Tillage Research* **94**, 295–304 (2007).
18. López-Fando, C. & Pardo, M. T. Changes in soil chemical characteristics with different tillage practices in a semi-arid environment. *Soil Tillage Res.* **104**, 278–284 (2009).
19. Zhao, X. *et al.* Responses of soil pH to no-till and the factors affecting it: a global meta-analysis. *Global Change Biol.* **28**, 154–166 (2022).
20. Zuber, S. M. & Villamil, M. B. Meta-analysis approach to assess effect of tillage on microbial biomass and enzyme activities. *Soil Biol. Biochem.* **97**, 176–187 (2016).
21. Li, Y. *et al.* Effect of no-tillage on soil bacterial and fungal community diversity: a meta-analysis. *Soil Tillage Res.* **204**, 104721 (2020).
22. Siles, J. A. *et al.* Long-term restoration with organic amendments is clearer evidenced by soil organic matter composition than by changes in microbial taxonomy and functionality. *Appl. Soil Ecol.* **198**, 105383 (2024).
23. Abdalla, K. *et al.* Long-term continuous farmyard manure application increases soil carbon when combined with mineral fertilizers due to lower priming effects. *Geoderma* **428**, 116216 (2022).
24. Okolo, C. C. *et al.* Priming effect in semi-arid soils of northern Ethiopia under different land use types. *Biogeochemistry* **158**, 383–403 (2022).
25. Fang, Y.-Y., Nazaries, L., Singh, B. K. & Singh, B. P. Microbial mechanisms of carbon priming effects revealed during the interaction of crop residue and nutrient inputs in contrasting soils. *Global Change Biology* **24**, 2775–2790 (2018).
26. Song, X.-J. *et al.* Positive priming effect explained by microbial nitrogen mining and stoichiometric decomposition at different stages. *Soil Biol. Biochem.* **175**, 108852 (2022).
27. Zhang, Q. *et al.* Nitrogen addition stimulates priming effect in a subtropical forest soil. *Soil Biology and Biochemistry* **160**, 108339 (2021).

ARTICLE IN PRESS

ARTICLE IN PRESS

28. Zhang, W.-D., Wang, X.-F. & Wang, S.-L. Addition of external organic carbon and native soil organic carbon decomposition: a meta-analysis. *PLOS One* **8**, e54779 (2013).
29. De Nobili, M., Contin, M., Mondini, C. & Brookes, P. C. Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biology and Biochemistry* **33**, 1163–1170 (2001).
30. Kuzyakov, Y. & Bol, R. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biology and Biochemistry* **38**, 747–758 (2006).
31. Rodrigues, L. A. T. *et al.* Carbon sequestration capacity in no-till soil decreases in the long-term due to saturation of fine silt plus clay-size fraction. *Geoderma* **412**, 115711 (2022).
32. Dijkstra, F. A., Carrillo, Y., Pendall, E. & Morgan, J. A. Rhizosphere priming: a nutrient perspective. *Frontiers in microbiology* **4**, 216 (2013).
33. Wang, C., Zhu, B., Luo, Y. & Kuzyakov, Y. 100 years of soil organic matter priming research. *Global Change Biol.* **32**, e70674 (2026).
34. Chen, S. *et al.* Deciphering the active bacteria involving glucose-triggered priming effect in soils with gradient N inputs. *Soil Biol. Biochem.* **199**, 109612 (2024).
35. Chen, R.-R. *et al.* Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Global Change Biology* **20**, 2356–2367 (2014).
36. Fontaine, S. *et al.* Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil biology and Biochemistry* **43**, 86–96 (2011).
37. Dimassi, B. *et al.* Effect of nutrients availability and long-term tillage on priming effect and soil C mineralization. *Soil Biology and Biochemistry* **78**, 332–339 (2014).
38. Sarker, J. R. *et al.* Tillage history and crop residue input enhanced native carbon mineralisation and nutrient supply in contrasting soils under long-term farming systems. *Soil and Tillage Research* **193**, 71–84 (2019).
39. Bol, R., Moering, J., Kuzyakov, Y. & Amelung, W. Quantification of priming and CO_2 respiration sources following slurry-C incorporation into two grassland soils with different C content. *Rapid Comm Mass Spectrometry* **17**, 2585–2590 (2003).

ARTICLE IN PRESS

ARTICLE IN PRESS

40. Blagodatskaya, E. & Kuzyakov, Y. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biol. Fertil. Soils* **45**, 115–131 (2008).

41. Fontaine, S. *et al.* Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **450**, 277–280 (2007).

42. Stott, D. E., Andrews, S. S., Liebig, M. A., Wienhold, B. J. & Karlen, D. L. Evaluation of β-Glucosidase Activity as a Soil Quality Indicator for the Soil Management Assessment Framework. *Soil Science Soc of Amer J* **74**, 107–119 (2010).

43. Chen, J. *et al.* Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Global Change Biol.* **23**, 1328–1337 (2017).

44. Allen, A. S. & Schlesinger, W. H. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. *Soil Biology and Biochemistry* **36**, 581–589 (2004).

45. Demoling, F., Figueroa, D. & Bååth, E. Comparison of factors limiting bacterial growth in different soils. *Soil Biology and Biochemistry* **39**, 2485–2495 (2007).

46. Liang, Y. *et al.* Stoichiometry regulates rice straw-induced priming effect: The microbial life strategies. *Soil Biology and Biochemistry* **196**, 109514 (2024).

47. Whitman, T. *et al.* Dynamics of microbial community composition and soil organic carbon mineralization in soil following addition of pyrogenic and fresh organic matter. *The ISME journal* **10**, 2918–2930 (2016).

48. Zhang, Y. *et al.* Spatio-temporal microbial regulation of aggregate-associated priming effects under contrasting tillage practices. *Science of the Total Environment* **925**, 171564 (2024).

49. Gioacchini, P. *et al.* Influence of urease and nitrification inhibitors on N losses from soils fertilized with urea. *Biology and fertility of soils* **36**, 129–135 (2002).

50. Qiu, Q., Wu, L., Ouyang, Z., Li, B. & Xu, Y. Different effects of plant-derived dissolved organic matter (DOM) and urea on the priming of soil organic carbon. *Environmental Science: Processes & Impacts* **18**, 330–341 (2016).

ARTICLE IN PRESS

ARTICLE IN PRESS

51. Buckeridge, K. M., Creamer, C. & Whitaker, J. Deconstructing the microbial necromass continuum to inform soil carbon sequestration. *Funct. Ecol.* **36**, 1396–1410 (2022).
52. Liu, Q. *et al.* Bacterial necromass decomposition and priming effects in paddy soils depend on long-term fertilization. *Soil Biol. Biochem.* **211**, 109992 (2025).
53. Six, J. & Paustian, K. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biol. Biochem.* **68**, A4–A9 (2014).
54. You, M. *et al.* Effect of reduction of aggregate size on the priming effect in a mollisol under different soil managements. *Eur. J. Soil Sci.* **70**, 765–775 (2019).
55. Tian, J. *et al.* Aggregate size and their disruption affect 14C-labeled glucose mineralization and priming effect. *Appl. Soil Ecol.* **90**, 1–10 (2015).
56. Fernandez-Ugalde, O. *et al.* LUCAS 2018 soil module. *Presentation of dataset and results, EUR* **31144**, (2022).
57. Brodie, C. R. *et al.* Evidence for bias in C/N, δ13C and δ15N values of bulk organic matter, and on environmental interpretation, from a lake sedimentary sequence by pre-analysis acid treatment methods. *Quaternary Science Reviews* **30**, 3076–3087 (2011).
58. Agriculture, U. S. D. of. *Soil Survey Manual (U.S. Department of Agriculture Handbook No. 18)*. (Lulu.com, 2019).
59. Le Bissonnais, Y. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. *European J Soil Science* **47**, 425–437 (1996).
60. ISO. Soil Quality: Measurement of the Stability of Soil Aggregates Subjected to the Action of Water. (2012).
61. AFNOR. Soil quality – physical methods – measuring of the bulk density of undisturbed soil samples – cylinder method. (1992).
62. Trasar-Cepeda, M. C., Gil Sotres, F. & Guitián Ojea, F. Determinación de la actividad de la fosfatasa en suelos gallegos. Precisiones al método de Saratchandra y Perrott. *Anal. Edafol. Agrobiol* **44**, 987–991 (1985).

ARTICLE IN PRESS

ARTICLE IN PRESS

63. Tabatabai, M. A. & Bremner, J. M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**, 301–307 (1969).

64. Eivazi, F. & Tabatabai, M. A. Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry* **20**, 601–606 (1988).

65. Saa, A., Trasar-Cepeda, M. C., Gil-Sotres, F. & Carballas, T. Changes in soil phosphorus and acid phosphatase activity immediately following forest fires. *Soil Biology and Biochemistry* **25**, 1223–1230 (1993).

66. Vuorinen, A. H. Requirement of p-nitrophenol standard for each soil. https://www.cabidigitallibrary.org/doi/full/10.5555/19931976399 (1993).

67. Nannipieri, P., Ceccanti, B., Cervelli, S. & Matarese, E. Extraction of Phosphatase, Urease, Proteases, Organic Carbon, and Nitrogen from Soil. *Soil Science Soc of Amer J* **44**, 1011–1016 (1980).

68. Nannipieri, P., Pedrazzini, F., Arcara, P. G. & Piovanelli, C. Changes in amino acids, enzyme activities, and biomasses during soil microbial growth. *Soil Science* **127**, 26–34 (1979).

69. Schutter, M. E. & Dick, R. P. Comparison of Fatty Acid Methyl Ester (FAME) Methods for Characterizing Microbial Communities. *Soil Science Society of America Journal* **64**, 1659–1668 (2000).

70. Vera, A., Bastida, F., Patiño-García, M. & Moreno, J. L. The effects of boron-enriched water irrigation on soil microbial community are dependent on crop species. *Applied Soil Ecology* **181**, 104677 (2023).

71. Dungait, J. A. J. *et al.* Variable responses of the soil microbial biomass to trace concentrations of 13C-labelled glucose, using 13C-PLFA analysis. *European Journal of Soil Science* **62**, 117–126 (2011).

72. Frostegård, Å., Bååth, E. & Tunlio, A. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry* **25**, 723–730 (1993).

73. Brant, J. B., Sulzman, E. W. & Myrold, D. D. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. *Soil Biology and Biochemistry* **38**, 2219–2232 (2006).

74. Rinnan, R. & Bååth, E. Differential Utilization of Carbon Substrates by Bacteria and Fungi in Tundra Soil. *Appl Environ Microbiol* **75**, 3611–3620 (2009).

ARTICLE IN PRESS

75. Schloss Patrick D. *et al.* Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541 (2009).

76. Callahan, B. J. *et al.* DADA2: high-resolution sample inference from illumina amplicon data. *Nat. Methods* **13**, 581–583 (2016).

77. Bastida, F., Torres, I. F., Hernández, T. & García, C. The impacts of organic amendments: Do they confer stability against drought on the soil microbial community? *Soil Biology and Biochemistry* **113**, 173–183 (2017).

78. Moreno, J. L., Torres, I. F., García, C., López-Mondéjar, R. & Bastida, F. Land use shapes the resistance of the soil microbial community and the C cycling response to drought in a semi-arid area. *Science of the Total Environment* **648**, 1018–1030 (2019).

79. Sáez-Sandino, T. *et al.* Increasing numbers of global change stressors reduce soil carbon worldwide. *Nat. Clim. Change* **14**, 740–745 (2024).

80. Yang, R.-M. *et al.* Comparison of boosted regression tree and random forest models for mapping topsoil organic carbon concentration in an alpine ecosystem. *Ecological indicators* **60**, 870–878 (2016).

81. Archer, E. EricArcher/rfPermute. (2024).

82. Lefcheck, J., Byrnes, J. & Grace, J. Package 'piecewiseSEM'. *R package version* **1**, (2016).

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AUTHOR COTRIBUTIONS STATEMENT

F.B and X.D conceived the study and obtained funding. X.D., A.V, M.P, J.A.S, W.Z, M.d.B and G.Z conducted experimental analyses and processed data and models. C.A. and S.M provided funding. C.A., E.T., I.S., M.A.P, F.V, SDD, C.T., A.P.F., A.E., TvV., M.F.D., M.v.H., R.W., G.P., I.D., J.Z., R.M., M.S., A.G., C.R., R.M., M.V., M.B provided soil samples and background data for modelling. F.B and X.D. wrote the manuscript with inputs from all co-authors. All coauthors contributed to the writing and review.

Authors declare no conflict of interest

FIGURE CAPTIONS

Figure 1. Importance of multiple ecological factors on soil organic carbon. Multi-model inference analyses suggest the importance of multiple factors such as priming on SOC (**A**). Colored columns indicate significant variables. The Bayesian Information Criterion (BIC) provides a measure of model adequacy, with lower values indicating better fit. The delta column shows the BIC difference relative to the best model, with values <4 suggesting performance comparable to the top-ranked model. Model weights indicate the relative contribution of each top model to the averaged model. The bar graphs illustrate the relative importance of each predictor, expressed as the percentage of explained variance (**B**). TSEA (temperature seasonality); BD (bulk density).

Figure 2. **The soil priming effect (PE) across management intensities, fertilization types and tillage conditions**. Box plots illustrating the PE after 32 days of incubation, comparing (a) management intensities (L: Low intensity; ML: Medium-low intensity; MH: Medium-high intensity; H: High intensity), (b) fertilization types (MN: mineral fertilization; OG: organic fertilization), and (c) tillage intensities (RT: reduced tillage; ST: standard tillage; NT: no-tillage). *n* as follows: Fertilization: Mineral = 68, Organic = 24; Tillage: RT = 31, ST = 37, NT = 24; Intensity: L = 14, ML = 31, MH = 20, H = 27. Different letters above each box denote the significance of differences at $P < 0.05$ (two-sided Fisher's LSD-test). Exact *P* values for the overall group comparisons are shown in each panel. The boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the vertical line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. Dots represent individual measured values.

Figure 3. Modelling the soil priming effect (PE). (a) Random Forest mean predictor importance (% increase in MSE (mean square error)) of selected variables, assessing PE across different management intensities in soils across Europe (n = 92). Predictors belonging to the same category were represented with the same color according to the legend. Significance in panel (a) was assessed using rfPermute permutation-derived *P* values for %IncMSE (299 permutations), without additional adjustment for multiple comparisons; exact *P* values are provided in Supplementary Table 3. (b) Standardized total

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effects (STE) of the selected factors on the PE derived from the structural equation model (SEM). This is the sum of the direct and indirect effects of each predictor on PE. Significance in panel (b) was assessed from the piecewise SEM using two-sided tests from the component linear models, without additional adjustment for multiple comparisons; exact *P* values are provided in Supplementary Table 3. (c) Results of the SEM assessing the direct and indirect effects of the studied factors on the PE. Numbers adjacent to arrows and arrow sizes reflect the strength of the effect. Path significance in panel (c) was evaluated using two-sided tests from the component linear models in the piecewise SEM. Model fit statistics are shown in the panel (Fisher's $C = 11.185$; $P = 0.513$; df = 12; AIC = 521.222). Asterisks indicate significance levels (*$P < 0.05$, **$P < 0.01$, and *$P < 0.001$). SOC: soil organic carbon; TN: total nitrogen; P: phosphorus; BG: β-glucosidase; ACP: acid phosphomonoesterase; ALP: alkaline phosphomonoesterase; BD: Bulk density; MWD: aggregate stability; df, degree of freedom; AIC, Akaike information criterion; BIC, Bayesian information criterion.

Figure 4. Selected dependences of the soil priming effect (PE) on soil properties evaluated by regression analyses. (a) PE versus soil organic carbon (SOC). (b) PE versus total nitrogen (TN). (c) PE versus clay. (d) PE versus C/P ratio. (e) PE versus urease activity. (f) PE versus aggregate stability (MWD). Relationships were assessed using simple linear regression analyses with two-sided tests, without additional adjustment for multiple comparisons. R^2 and *P* values are shown for each regression analysis. Shaded areas represent 95% confidence intervals for the regression lines. L: Low intensity; ML: Medium-low intensity; MH: Medium-high intensity; H: High intensity.

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Editor's Summary

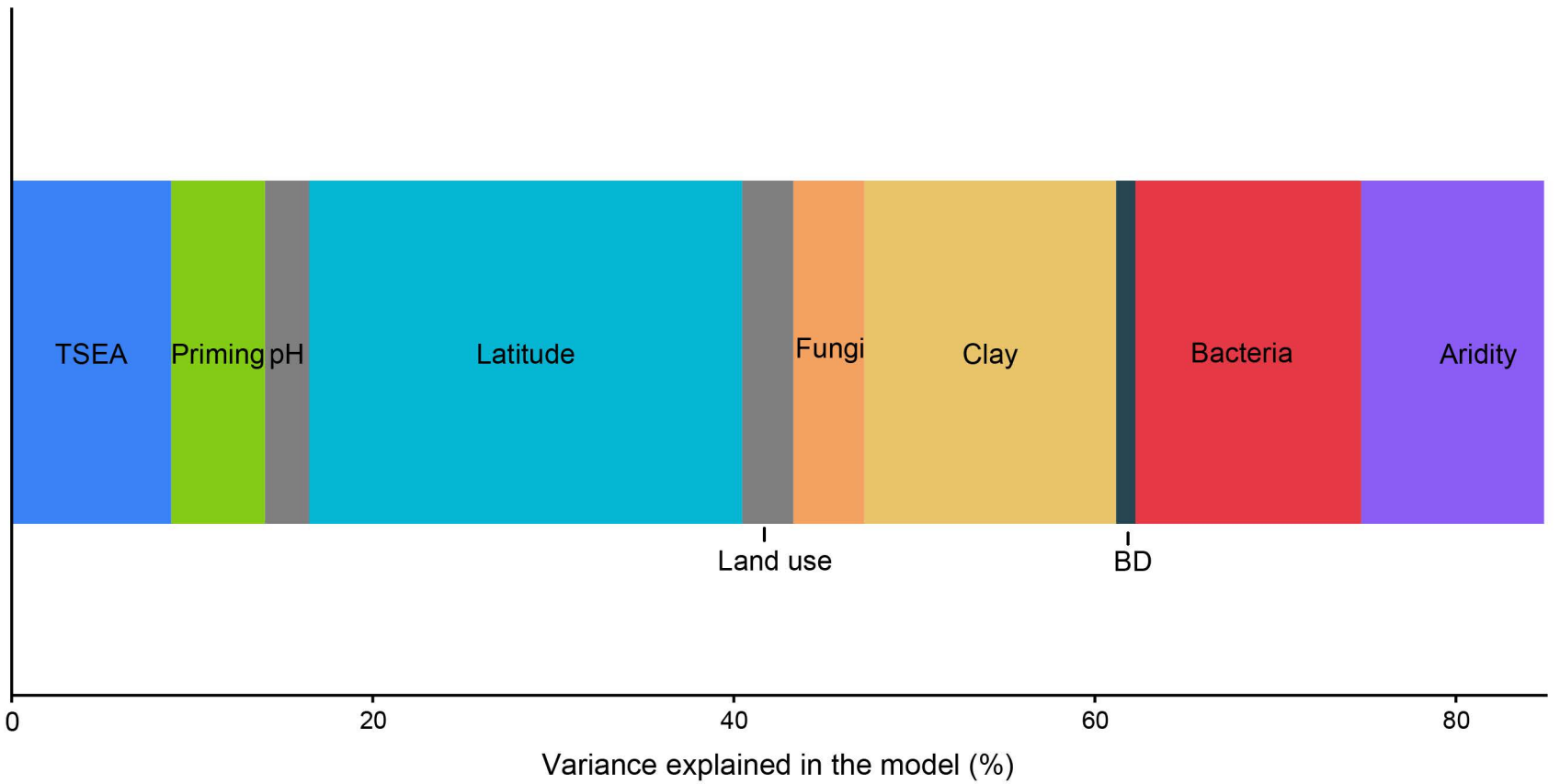
The priming effect (PE) predicts soil carbon across croplands. Using soils from European long-term experiments, we show that low-intensity management enhances PE, whereas intensive fertilization and tillage reduce it.

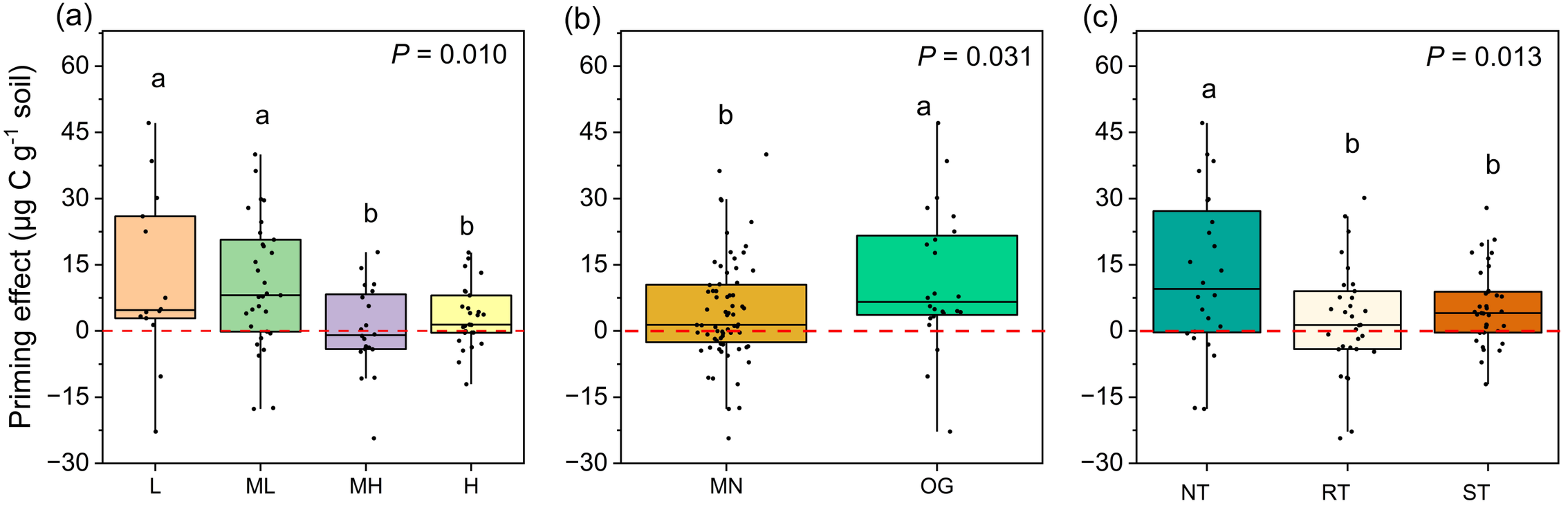
Peer review information: *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work. A peer review file is available.

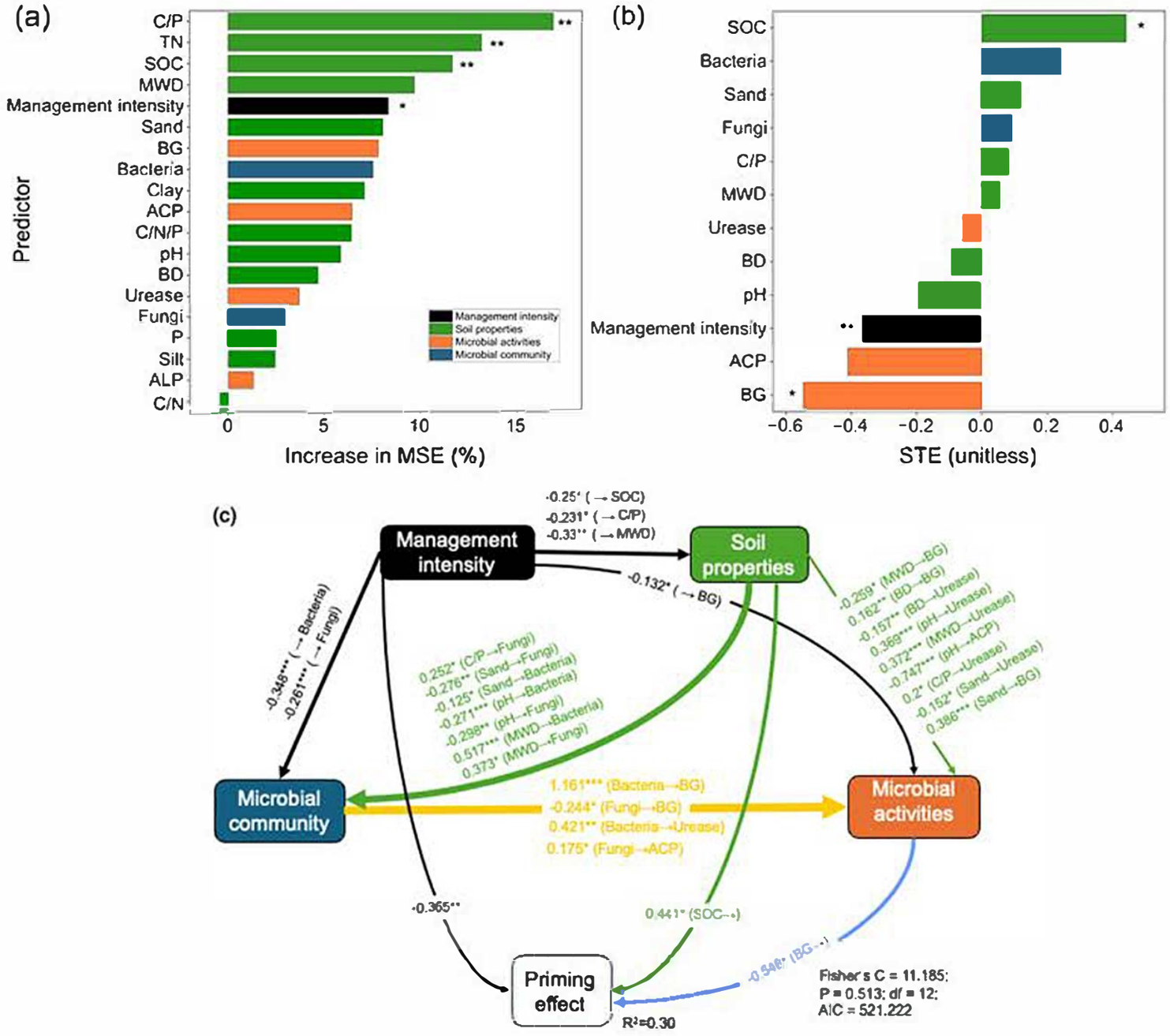
(A)

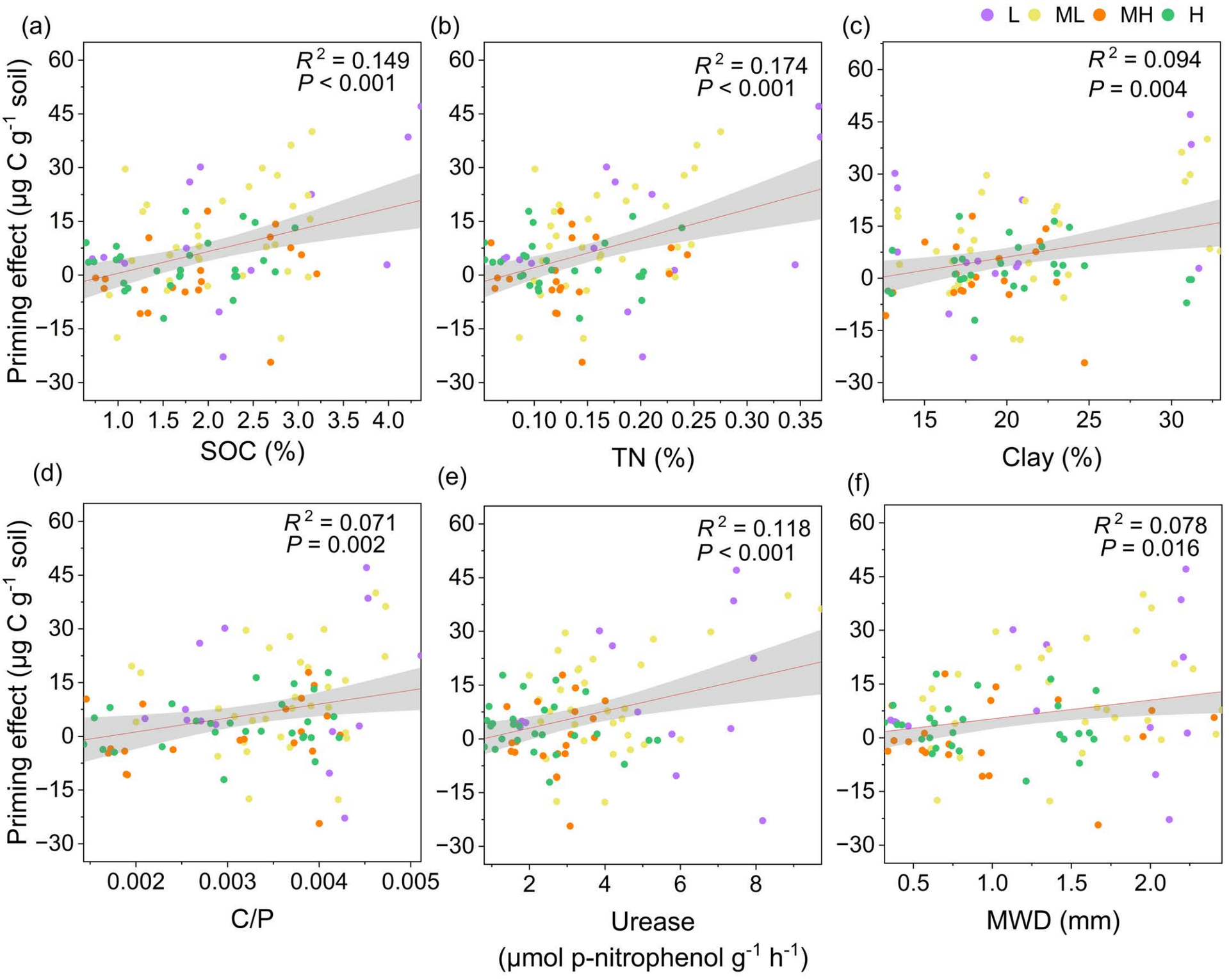
Survey	Aridity	Bacteria	BD	Clay	Fungi	Land use	Latitude	MAT	Priming	pH	PSEA	TSEA	df	LogLik	BIC	Delta	Weight
LTEs													10	-6.73	58.68	0.00	0.36
													9	-9.27	59.23	0.55	0.27
													10	-7.82	60.85	2.17	0.12
													11	-5.92	61.58	2.90	0.08
													11	-6.19	62.13	3.45	0.06
													10	-8.71	62.64	3.96	0.05
													11	-6.47	62.67	3.99	0.05

(B)




(a)
P = 0.010
Priming effect (μg C g-1 soil)
60
45
30
15
0
−15
−30
a
a
b
b
L
ML
MH
H
(b)
P = 0.031
b
a
MN
OG
(c)
P = 0.013
a
b
b
NT
RT
ST


(a)
C/P
TN
SOC
MWD
Management intensity
Sand
BG
Bacteria
Clay
ACP
C/N/P
pH
BD
Urease
Fungi
P
Silt
ALP
C/N
Predictor
Management intensity
Soil properties
Microbial activities
Microbial community
0
5
10
15
Increase in MSE (%)
(b)
SOC
Bacteria
Sand
Fungi
C/P
MWD
Urease
BD
pH
Management intensity
ACP
BG
−0.6
−0.4
−0.2
0.0
0.2
0.4
STE (unitless)
(c)
-0.25* (→ SOC)
-0.231* (→ C/P)
-0.33** (→ MWD)
Management intensity
Soil properties
-0.132* (→ BG)
-0.348*** (→ Bacteria)
-0.261*** (→ Fungi)
0.252* (C/P→Fungi)
-0.276** (Sand→Fungi)
-0.125* (Sand→Bacteria)
-0.271*** (pH→Fungi)
-0.298** (pH→Bacteria)
0.517*** (MWD→Bacteria)
0.373* (MWD→Fungi)
-0.259* (MWD→BG)
0.162** (BD→BG)
-0.157** (BD→Urease)
0.369*** (pH→Urease)
0.372*** (MWD→ACP)
-0.747*** (pH→ACP)
0.2* (C/P→Urease)
-0.152* (Sand→Urease)
0.386*** (Sand→BG)
Microbial community
1.161*** (Bacteria→BG)
-0.244* (Fungi→BG)
0.421** (Bacteria→Urease)
0.175* (Fungi→ACP)
Microbial activities
-0.365**
0.441* (SOC→)
-0.548* (BG→)
Priming effect
R²=0.30
Fisher's C = 11.185;
P = 0.513; df = 12;
AIC = 521.222


(a)
(b)
(c)
(d)
(e)
(f)
L
ML
MH
H
R^2 = 0.149
P < 0.001
R^2 = 0.174
P < 0.001
R^2 = 0.094
P = 0.004
R^2 = 0.071
P = 0.002
R^2 = 0.118
P < 0.001
R^2 = 0.078
P = 0.016
Priming effect (μg C g^{-1} soil)
SOC (%)
TN (%)
Clay (%)
C/P
Urease
(μmol p-nitrophenol g^{-1} h^{-1})
MWD (mm)