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Priming effects in soils across Europe

José A. Siles[1], Marta Díaz[1], Alfonso Vera[1], Nico Eisenhauer[2,3], Carlos A. Guerra[2,4], Linnea C. Smith[2,4], François Buscot[5,4,2], Thomas Reitz[5,2], Claudia Breitkreuz[5], Johan van den Hoogen[6], Thomas W. Crowther[6], Alberto Orgiazzi[7], Yakov Kuzyakov[8,9,10], Manuel Delgado-Baquerizo[11], Felipe Bastida[1,*]

[1]Department of Soil and Water Conservation and Waste Management, CEBAS-CSIC, Murcia, Spain.

[2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

[3]Institute of Biology, Leipzig University, Leipzig, Germany

[4]Institute of Biology, Martin Luther University Halle Wittenberg, Halle (Saale), Germany

[5]Soil Ecology Department, UFZ - Helmholtz Centre for Environmental Research, Halle (Saale), Germany

[6]Department of Environmental Systems Science, Institute of Integrative Biology, ETH Zürich, Zurich, Switzerland

[7]European Commission, Joint Research Centre, Ispra, Italy

[8]Department of Soil Science of Temperate Ecosystems and Department of Agricultural Soil Science, University of Gottingen, Gottingen, Germany

[9]Agro-Technological Institute, RUDN University, Moscow, Russia

[10]Institute of Environmental Sciences, Kazan Federal University, Kazan, Russia

[11]Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo de Olavide, Sevilla, Spain

*Corresponding author. Email: fbastida@cebas.csic.es

Priming effects in soils across Europe

Running title: Soil priming effects across Europe

José A. Siles[1], Marta Díaz-López[1], Alfonso Vera[1], Nico Eisenhauer[2,3], Carlos A. Guerra[2,4], Linnea C. Smith[2,4], François Buscot[5,4,2], Thomas Reitz[5,2], Claudia Breitkreuz[5], Johan van den Hoogen[6], Thomas W. Crowther[6], Alberto Orgiazzi[7], Yakov Kuzyakov[8,9,10], Manuel Delgado-Baquerizo[11], Felipe Bastida[1,*]

ORCID ID's: José A. Siles: 0000-0002-5850-0281; Marta Díaz-López: 0000-0002-4178-8047; Alfonso Vera: 0000-0002-1822-0496; Nico Eisenhauer: 0000-0002-0371-6720; Carlos A. Guerra: 0000-0003-4917-2105; Linnea C. Smith: 0000-0002-3038-0580; François Buscot: 0000-0002-2364-0006; Thomas Reitz: 0000-0001-6581-6316; Claudia Breitkreuz: 0000-0003-1317-4942; Johan van den Hoogen: 0000-0001-6624-8461; Thomas W. Crowther: 0000-0001-5674-8913; Alberto Orgiazzi: 0000-0002-8390-6654; Yakov Kuzyakov: 0000-0002-9863-8461; Manuel Delgado-Baquerizo: 0000-0002-6499-576X; Felipe Bastida: 0000-0001-9958-7099.

[1]Department of Soil and Water Conservation and Waste Management, CEBAS-CSIC, Murcia, Spain.

[2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

[3]Institute of Biology, Leipzig University, Leipzig, Germany

[4]Institute of Biology, Martin Luther University Halle Wittenberg, Halle (Saale), Germany

[5]Soil Ecology Department, UFZ - Helmholtz Centre for Environmental Research, Halle (Saale), Germany

[6]Department of Environmental Systems Science, Institute of Integrative Biology, ETH Zürich, Zurich, Switzerland

[7]European Commission, Joint Research Centre, Ispra, Italy

[8]Department of Soil Science of Temperate Ecosystems and Department of Agricultural Soil Science, University of Gottingen, Gottingen, Germany

[9]Agro-Technological Institute, RUDN University, Moscow, Russia

[10]Institute of Environmental Sciences, Kazan Federal University, Kazan, Russia

[11]Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo de Olavide, Sevilla, Spain

*Corresponding author: Felipe Bastida. Email: fbastida@cebas.csic.es.

Abstract

Land use is a key factor driving changes in soil carbon (C) cycle and contents worldwide. The priming effect (PE) -CO_2 emissions from changed soil organic matter decomposition in response to fresh C inputs- is one of the most unpredictable phenomena associated with C cycling and related nutrient mobilization. Yet, we know very little about the influence of land use on soil PE across contrasting environments. Here, we conducted a continental-scale study to (i) determine the PE induced by ^{13}C-glucose additions to 126 cropland and seminatural (forests and grasslands) soils from 22 European countries; (ii) compare PE magnitude in soils under various crop types (i.e., cereals, non-permanent industrial crops and orchards); and (iii) model the environmental factors influencing PE. On average, PE were negative in seminatural (with values ranging between -60 and 26 μg C g^{-1} soil after 35 days of incubation; median = -11) and cropland (from -55 to 27 μC g^{-1} soil; median = -4.3) soils, meaning that microbial communities preferentially switched from soil organic C decomposition to glucose mineralization. PE were significantly less negative in croplands compared with seminatural ecosystems and not influenced by the crop type. PE were driven by soil basal respiration (reflecting microbial activity), microbial biomass C and soil organic C, which were all higher in seminatural ecosystems compared with croplands. This cross European experimental and modeling study elucidated that PE intensity is dependent on land use and allowed to clarify the factors regulating this important C cycling process.

Keywords: priming effect, land use, soil organic matter, carbon cycling, agroecosystems, land management

1. INTRODUCTION

Agricultural use of soils, which is expected to keep increasing to support a larger human population, usually leads to a decline in soil organic carbon (SOC) contents (Guo and Gifford, 2002; Wei et al., 2014). SOC stocks result from the balance between C inputs from plants and/or fertilizers and C outputs through SOC and plant residue mineralization by microorganisms and are modified by stabilizing mechanisms and lateral fluxes (e.g., runoff of dissolved organic C) (Guenet et al., 2018). This sensitive balance between input and output fluxes depends, among other factors, on the priming effect (PE), defined as the change in the rate of SOC mineralization by microorganisms in response to fresh C inputs (Kuzyakov et al., 2000; Blagodatskaya et al., 2007).

The PE regulates SOC decomposition at microbial scale, having consequences on soil profile, field, regional and global scales, and can result in increasing (positive PE) or decreasing (negative PE) rates of SOC mineralization (Blagodatskaya and Kuzyakov, 2008). A positive PE occurs when fresh substrate induces microbial growth and/or stimulates microbial enzyme production (i.e., microbial activation mechanism), which leads to a more intense SOC mineralization (Blagodatskaya et al., 2014). A negative PE occurs because of the preferential substrate utilization hypothesis; i.e., microorganisms decompose the newly added C sources to a higher extent than the SOC because of their greater availability (Blagodatskaya et al., 2007). The PE magnitude is highly variable among soils, with values of native SOC decomposition ranging from a decrease by 95% to a stimulation by 1207% (Zhang et al., 2013; Perveen et al., 2019). Previous studies have identified factors related to climate (temperature and moisture), plant properties (quantity and quality of fresh C input), soil characteristics (texture, SOC content and stability and pH) and microbial communities (biomass, community composition and activity) as drivers of the PE at local and regional scales (Fontaine et al., 2003; Pascault et al., 2013; Cheng et al., 2014; Razanamalala et al., 2018; Chen et al., 2019; Feng and Zhu, 2021). However, unlike for natural ecosystems, the impact of agricultural use on the PE is far less understood, which hampers our capacity to predict how increases in cropping area will influence C stocks at a large spatial scale. In light of the ongoing discussion on the post-2020 European Common Agricultural Policy and the need to promote soil management practices that contribute to C sequestration (Pe'er et al., 2019), the impact of the PE on terrestrial CO_2 emissions and global warming has to be taken into account as an important factor for developing more sustainable agroecosystems.

In the context of land use (i.e., natural *vs*. croplands), the implications of the PE are important, yet poorly determined, because they may decrease soil C stocks and alter the balance of C fluxes between soils and the atmosphere (Guenet et al., 2018). For instance, sustainable agricultural practices frequently involve approaches such as the application of C-rich materials (i.e., organic amendments) to increase SOC contents. However, a strong positive PE induced by the labile C sources in these organic amendments may increase the rates of soil CO_2 release and have a positive feedback on global warming, hampering the suitability of such practices (Thangarajan et al., 2013; Shahbaz et al., 2017; Chen et al., 2019; Guttières et al., 2021). The understanding of the direction and extent of the PE, as well as its predictors and its interactions with other environmental factors, is thus crucial for a proper comprehension of how land uses impact C cycling. To gain insights into this matter, studies assessing the PE in soils with contrasting land uses and with broad range of chemical, physical and microbial properties, but under the same experimental conditions, are more accurate than meta-analyses working on data from experiments not always methodologically comparable (Perveen et al., 2019). Previous local studies of the PE in cropland soils in comparison with other land uses have shown contradictory results (Moreno et al., 2019; Perveen et al., 2019; Guttières et al., 2021), which points to uncertainties on whether and how land use-mediated differences in soil properties (e.g., pH, SOC content, water retention, microbial communities, etc.) drive the PE (Rodrigues et al., 2013; Finn et al., 2017). Thus, it is not clear if the local factors regulating PE are similar at continental scale. Studies evaluating the PE in a broad range of soils subjected to agricultural and natural land uses and from a large geographical scale are, therefore, essential to get a unified ecological context and an integrative approach to understand this phenomenon.

Here, we aimed to fill out important knowledge gaps by (i) quantifying and comparing the PE in soils from cropland and seminatural (i.e., forests and grasslands) ecosystems, as well as in cropland soils cultivated with three important crop types in Europe: cereals, non-permanent industrial crops, and permanent fruit and vineyard orchards; and (ii) modelling the main factors controlling the PE under contrasting land uses. To address these knowledge gaps, PE were measured after ^{13}C-glucose addition in soils sampled from a large variety of cropland and seminatural (grasslands and forests) ecosystems from 126 locations across 22 European countries, covering three broad climate regimes: semiarid, temperate and cold. The data obtained were then subjected to Random Forest analysis and structural equation modelling (SEM) to explore the main drivers of the PE. Our initial hypothesis was that positive PE are common in soils from both seminatural and cropland ecosystems, being the magnitude of the phenomenon more intense in croplands. The differences in PE between land uses would be mediated by (i) the lower contents of SOC and microbial activity (e.g., respiration rates) under croplands (relative to forests and grasslands); and (ii) the adaptation of soil microorganisms in croplands to quickly respond to C inputs in terms of growth and activity (r-strategists) due to their strong limitation by available C pools and their consequent starvation by C and energy (Young and Ritz, 2000; Szoboszlay et al., 2017). The rapid (in a matter of days) growth and activation of microorganisms in response to the addition of glucose would further accelerate SOC mineralization in croplands. In contrast, greater SOC content and availability in natural ecosystems (i.e., northern and temperate forests, grasslands, etc.) would reduce the sensitivity of microbial communities to labile C inputs, reducing the PE magnitude.

2. MATERIALS AND METHODS

2.1 Soil Sampling

Soil samples were collected from a total of 126 locations in 22 EU member states under the auspices of the 2018 “Land Use/Cover Area frame statistical Survey” -LUCAS- (Orgiazzi et al., 2018). To assess the PE in ecosystems with contrasting land use, croplands were compared with more natural land uses such as forests and grasslands. Considering that most grasslands and forests are also not fully natural, have been managed over decades or centuries and are affected by global change, we grouped forests and grasslands into the category of seminatural. This decision was also based on the fact that there were no significant differences (p>0.05) in the PE between forest and grassland soils across Europe probably as a consequence of the lack of significant differences in basal respiration, microbial biomass C (MBC) and SOC between these two land uses (Fig. S1). Samples from 86 croplands and 40 seminatural sites (24 and 16 samples from forest and grassland ecosystems, respectively) were selected considering three broad climate types: semiarid, temperate and cold. Mean annual temperature (MAT) ranged between 0.6 and 20.8 °C, and aridity index (AI) between 0.20 and 1.74. Further, crop types were classified into three categories: cereals (CER; barley, wheat and maize; 48 soil samples), non-permanent industrial crops (OIL; sunflower and rape; 14 soil samples), and woody orchard crops (ORC; olive groves and vineyards, pear and apple orchards; 24 soil samples). Details about location, land use and predominant vegetation of each sampling site are presented in Table S1. Each sample was a composite of five subsamples from the top 20 cm of soil: four subsamples orthogonally collected in a 2-m radius from a central subsample. More details about soil sampling strategy and LUCAS can be found in Orgiazzi et al. (2018). After sampling, the soils were sieved (2 mm), air dried and stored at -20 °C.

2.2 Characterization of soil samples

Soil physical and chemical properties (sand percentage, pH, SOC content and total phosphorus (P) and potassium (K) contents) were taken from the 2018 LUCAS dataset. Data on net primary productivity (NPP) were collected from MODIS (Running and Zhao, 2019), MAT from WorldClim 2 (Fick and Hijmans, 2017) and AI from CGIAR (Trabucco and Zomer, 2018). Activities of β-glucosidase (E.C. 3.2.1.21), cellulase (EC 3.2.1.4) and xylosidase (EC 3.2.1.37) in the investigated samples were analyzed as described elsewhere (Breitkreuz et al., 2021). MBC was measured in an O_2 microcompensation apparatus (Scheu, 1992) using the substrate-induced respiration method (Anderson and Domsch, 1978). Bacterial and fungal abundance in each soil sample was assessed through quantification of ester-linked fatty acid methyl esters (EL-FAMEs), hereafter FAMEs, as described by Schutter and Dick (2000). Briefly, FAMEs were methylated, neutralized and extracted from 3 g of soil using the appropriate reagents and subsequently analyzed with a Trace Gas Chromatograph Ultra (Thermo Scientific) fitted with a 60-m capillary column (SGE Analytical Science) as described by Vera et al. (2021). The fatty acids i15:0, a15:0, i16:0, i17:0, 16:1w7, cy17:0, cy19:0, 10Me16:0, and 10Me18:0 were used as markers of bacterial abundance (Dungait et al., 2011; Frostegård et al., 1993), while 18:2w6,9t and 18:2w6,9c were used as fungal markers (Brant et al., 2006; Rinnan and Bååth, 2009). All the raw data used for the characterization of the soil samples are available in the Table S1.

2.3. Analysis of basal respiration and PE in soil incubation experiment

The procedure followed for the soil incubation experiment was similar to that described by Bastida et al. (2019). Two parallel sets of microcosms each containing 1 g of soil at 50% of water-holding capacity were pre-incubated in 20-ml glass vials sealed with a rubber septum for 14 days at 20 °C in the dark. This pre-incubation was conducted to allow soil microbes to adapt to the adjusted temperature and moisture conditions after freezing. After the pre-incubation, microcosms were opened to refresh their atmosphere and to remove the accumulated CO_2. Subsequently, one of the two sets of microcosms was amended with ^{13}C-glucose (99 atom% U-^{13}C, Cambridge Isotope Laboratories, Tewksbury, Massachusetts, US) dissolved in sterile deionized water at a dose of 186 µg of glucose-C per gram of soil, while the other microcosm set was supplemented only with sterile deionized water and used to measure rates of soil basal respiration. This glucose amount was calculated as 50% of the median content in MBC of all the soil samples (Blagodatskaya and Kuzyakov, 2008). Glucose was selected as substrate since it is a major component of root exudates, the most abundant plant carbohydrate monomer and because most microorganisms can easily metabolize it. The 252 (2 series of 126 soil samples) microcosms were incubated for 35 days at 20 °C in the dark. According to Blagodatskaya and Kuzyakov (2008), this period is adequate to assess real PE and not only apparent PE. Under these incubation conditions, anaerobic conditions were not reached in the atmosphere of any of the microcosms: the concentrations of CO_2 in the vials ranged between 0.10 and 1.8% and those of O_2 did so between 19 and 21% (Table S2).

2.4. CO_2 measurements and calculations of PE

After incubation, 4 mL of headspace gas from each vial were transferred to pre-evacuated glass vials (Labco Limited, Lampeter, Wales, UK) and the quantity and isotopic composition of released CO_2 was determined. The $\delta^{13}C$ isotope analysis was performed using a Thermo Scientific GasBench-PreCon trace gas system coupled to a Delta V Plus IRMS (Thermo Scientific, Bremen, Germany) at the UC Davis Stable Isotope Facility as described by Bastida et al. (2017). The final delta values used for the ^{13}C calculations were expressed relative to international standards of V-PDB (Vienna Pee Dee Belemnite) as explained by Moreno et al. (2019). The $^{13}C/^{12}C$ isotopic ratio of CO_2 was used to calculate the percentage of CO_2 derived from the added glucose or from the soil. CO_2 amount produced in soils without ^{13}C-

glucose addition was used to measure basal respiration. The PE was defined as the increase or decrease in the CO_2 derived from the SOC mineralization following substrate addition compared to the control soil with water addition only. The PE was calculated as the total soil respiration following glucose addition minus the amount of C respired from the added ^{13}C-glucose and minus C respired from control soil without glucose addition (Moreno et al., 2019) (Eq. 1). This was expressed as the extra CO_2-C (µg C) released from soil.

$$PE = (total\ CO_2 - glucose\ derived\ CO_2) - total\ CO_2 \quad \text{(Eq. 1)}$$

The first part of the equation (total CO_2 – glucose derived CO_2) refers to the soil amended with glucose, and the second component (total CO_2) refers to the soil with water addition only. The PE was expressed as µg C g^{-1} soil and values ranging from -2 to +2 were considered as non-significant PE. Raw basal respiration, PE and glucose mineralization data for each sample are available in Table S1.

2.5. Statistical analyses

Non-parametric univariate PERMANOVA (Permutational Analysis of Variance) was used to check whether soil basal respiration, rates of glucose mineralization, PE and other variables differed significantly between land uses (croplands *vs*. seminatural) or among crop types. For this analysis, the *adonis* function in the R package *vegan* was used (Oksanen et al., 2013). Autocorrelation between variables was checked using the Durbin-Watson test. Data visualizations were performed using the R package *ggplot2* (Wickham, 2016) and CorelDRAW ver. 2020.

Random Forest analysis (Breiman, 2001) was conducted to identify the main predictors of variations in the soil PE as described by Delgado-Baquerizo et al. (2016). The list of predictors included land use (a categorical variable including the levels cropland and seminatural soils), environmental conditions (MAT, NPP, AI), soil physicochemical properties (sand proportion, pH and contents of SOC, P and K), microbial abundance (MBC and the contents of fatty acids representative for bacteria and fungi) and microbial activity (basal respiration, β-glucosidase, xylosidase and cellulase). The selection of these variables was made based on previous works highlighting that the dynamics of the PE are dependent on several of the abovementioned abiotic and biotic properties (Chen et al., 2014; Luo et al., 2016; Bastida et al., 2019; Chen et al., 2019). The importance (increase in mean square error percentage) and significance of each predictor was computed for each tree and averaged over the forest (999 trees) using the *rfPermute* package (Archer, 2020) of R. Significant factors of the PE were defined as those with $p<0.05$.

SEM (structural equation modeling) was used to build a detailed system-level understanding of the major direct and indirect effects of the aforementioned abiotic and biotic factors on the PE based on an *a priori* model (Fig. S2). SEM provides complementary insights into the factors that govern the soil PE under contrasting land-use types. Environmental factors (MAT, NPP and AI) were only used here as proxies of legacy effects, as incubations for the PE were done under controlled conditions, with constant soil water content and temperature. Since some of the variables were not normally distributed, the probability that a path coefficient differs from zero was tested using bootstrap tests. Bootstrapping tests in such cases do not assume that the data match a particular theoretical distribution. The goodness-of-fit of SEM model was checked using the Chi-square test, the root mean square error of approximation (RMSEA) and the Bollen–Stine bootstrap test (Schermelleh-Engel et al., 2003). Finally, to aid SEM final interpretation, we calculated the standardized total effects of land use and the remaining factors on the PE. The net influence that one variable has upon another is calculated by summing all direct and indirect pathways between the two variables. If the model fits the data well, the total effect should approximately be the bivariate correlation coefficient for that pair of variables.

3. RESULTS

3.1. Less negative PE in cropland soils

Cropland soils had significantly lower SOC contents, MBC and bacterial and fungal biomass than soils in seminatural environments, while the opposite was observed for pH (Fig. 1). Soils under croplands had approximately two times lower rates of basal respiration than those of seminatural sites (39 *vs*. 74 μg C g^{-1} soil, respectively, after 35 days of incubation; $p<0.001$; Fig. 2a). These results were concomitant with the percentages of glucose mineralization, which were significantly ($p=0.031$) higher in seminatural soils (Fig. S3a).

PE were significantly more negative ($p=0.002$) in seminatural soils (median = -11 μg C g^{-1} soil after 35 days) compared to those of croplands (-4.3 μg C g^{-1} soil) (Fig. 2b). In cropland soils, the PE ranged between -55 and 27 μC g^{-1} soil. Of the 86 cropland studied soils, 54 (~63% of the total) presented a negative PE, 21 soils had positive PE and the remaining 11 soils had no PE (PE of ±2 μg C g^{-1} soil were considered not different from 0). In seminatural soils, the PE ranged between -60 and 26 μg C g^{-1} soil. In this case, a negative PE was found in 30 (~75% of the total) of the 40 soils examined, 8 soils had positive PE, and 2 soils had no PE. Further, the rates of basal respiration, glucose mineralization and the PE were independent of cropland type (Fig. 2c, d and Fig. S3b).

3.2. Effects of agricultural uses on soil C and microbial communities drive PE

We used Random Forest to identify the factors controlling the PE in cropland and seminatural soils and SEM to understand the magnitude and direction of the relationships among the driving factors (see *a priori* SEM in Fig. S2). Random Forest showed that basal respiration and MBC were the most important (and significant) predictors of the PE (Fig. 3a). Complementary, our SEM (explaining more than half (i.e., 52%) of the PE variability) indicated that respiration and SOC are the significant drivers of PE (Fig. 3b). The model also showed that land use (as categorical variable) and basal respiration (reflecting microbial activity) were the only two factors having direct effects on the PE (Fig. 3c). Unexpectedly, environmental conditions did not exert a direct effect on PE. Land use (as categorical variable) directly influenced basal respiration, but also indirectly by inducing changes in MBC and SOC contents, fungal abundances and pH between cropland and seminatural soils. Therefore, according to our models, soil PE was directly influenced by basal respiration and indirectly by SOC and MBC, all of which are influenced by land use. In concordance, regression and Spearman correlation analyses indicated that the PE decreased with increase of basal respiration, MBC and SOC contents (Fig. 4 and Fig. S4). The Durbin-Watson statistic yielded values of around 2 for the regression analyses between respiration, MBC and SOC, which discards the autocorrelation between these factors. The activities of the enzymes β-glucosidase, cellulase and xylosidase were dependent on land use, AI, soil nutrient contents and microbial abundances (both FAME contents and MBC), but none of them were PE predictors, as revealed by Random Forest and SEM (Fig. 3). The contents of bacterial and fungal fatty acids increased with MBC (Fig. S4) and were directly and indirectly affected by land-use and environmental conditions, respectively.

4. DISCUSSION

Our study at continental scale provides unprecedented knowledge on the influence of land use in the capacity of soils to respond to fresh C inputs through the PE. The PE in croplands were less negative compared with those in seminatural soils. Consequently, the input of easily available C sources (glucose) decreased SOC mineralization, and this decrease was stronger in seminatural ecosystems compared to croplands. Accordingly, fresh organic C input contributes to the positive C balance not only by new SOC formation, but also by decreasing mineralization of existing SOC. MBC and SOC content as well as microbial activity -measured as basal respiration- were the main factors shaping the PE across European soils.

4.1. Negative PE across European soils and lack of differences across cropland types

According to the preferential utilization mechanism, the average negative PE in soils from both seminatural and cropland sites suggest that microorganisms switched from decomposition of hardly available SOC to the mineralization of the added glucose, at least during the first 35 days of incubation (Blagodatskaya et al., 2007). Negative PE occur when nutrients are sufficient and microbes do not need to mine soil organic matter to acquire them, indicating thus that microbes are mainly limited by available C (probably as a consequence of its recalcitrance) in most of the European soils (Guenet et al., 2010). Although the average PE was negative, high variability in PE magnitude and direction was common within each land use (Fig. 2). For example, 24% and 20% of the cropland and seminatural soils showed a positive PE, respectively. The most frequently reported response to glucose inputs is an acceleration in SOC mineralization (Paterson and Sim, 2013; Liu et al., 2017; Guttières et al., 2021); i.e., positive PE. However, some studies have reported that glucose addition to soil may lead to a negative PE (Kuzyakov and Bol, 2006). In this line, Qiao et al. (2014) argued that the occurrence of the PE requires the amount of added substrate to reach a specific threshold. We are thus aware of the limitations of using a sole glucose amount, but this was equivalent to the median content of MBC of all the tested soils. This glucose amount should have been sufficient to foster enzyme production and cause a SOC mineralization (i.e., a positive PE) without excessive microbial growth (Blagodatskaya and Kuzyakov, 2008). Nevertheless, we cannot rule out that using a specific ^{13}C-glucose dose for each soil equivalent to a given percentage of

MBC would have influenced our results. In this line, Blagodatskaya and Kuzyakov (2008) reported that if the amounts of added substrate C are lower than 15% of MBC, the magnitude of the PE linearly increases with the amount of added C. Instead, when the amount of added substrate-C exceeds 50% of MBC, the PE decreases with increasing amounts of added C. At the rates of substrate C exceeding 200–500% of MBC, the PE tends to be zero or even negative. We are also aware that the incubation temperature used (+20 ºC) is close to the MAT of some soils, but far from that of other soils. Since the incubation of each soil at its MAT was not feasible due to the high number of soils considered, we selected a temperature of 20 ºC because it is within the range of maximum and minimum temperatures for most of the soils. Increasing temperature has been shown to increase (Li et al., 2017), decrease (Yanni et al., 2017) or not affect the PE (Vestergård et al., 2016), without deep mechanistic explanations of these results (Guttierres et al., 2021). Despite these limitations (in common with most of studies on the PE) we considered that applying the same experimental conditions to all the soils was an appropriate approach to address our objective: assessing the effects of land use change and varying crop types on soil PE and elucidating its driving factors. Therefore, although our results may depend on the experimental conditions, it is important to highlight that a negative PE is a more common phenomenon than previously thought, which has important implications for predictions of SOC stocks and soil C fluxes under contrasting land uses (Tian et al., 2015). In this way, our results would be suggesting that addition of labile C sources to soils across Europe produces a slowdown in the decomposition rates of native SOC. This may result in a positive feedback on SOC stocks and buffer the increased rates of SOC mineralization related to the current conditions of global warming (Chen et al., 2019; Perveen et al., 2019; Liu et al., 2020), being this effect more pronounced in forest and grassland soils since they present more negative PE. However, these interpretations need to be considered with caution as PE assessed under laboratory conditions provides limited insights into the PE happening in nature because the variability in the frequency, magnitude and composition of soil C inputs, soil structure and climatic conditions occurring in real conditions are difficult to reproduce in the laboratory (Bastida et al., 2019; Zhou et al., 2021).

The differential management of croplands, such as cereals, industrial crops and fruit trees (regarding fertilization, irrigation, tillage, etc.), impacts soil physical and chemical properties –breaking aggregates, root exudation amount and composition, nutrient contents, changing oxygen diffusion, etc.–

(Li et al., 2021) and ultimately influence the activity of soil microbial communities and the PE (de Graaff et al., 2014; Panettieri et al., 2020). In line with this argumentation, we expected the PE magnitude in croplands to be dependent on crop type. However, there were no significant changes in the PE across cropland types (cereals, non-permanent industrial crops and orchards) and these results could be attributed to the absence of important differences in soil chemical and physical properties among cropland types (Fig. S5), contrary to our initial hypothesis. These findings are likely related to two facts: i) that conversion of natural ecosystems (having very broad range of soil conditions) to croplands always leads to convergence of soil properties (especially pH) narrowing their variability (Kuzyakov and Zamanian, 2019); and ii) the wide heterogeneity of agricultural practices, climate and soil conditions across Europe. For instance, the different phenology or status of the plant development at the time of soil sampling across crops could influence the observed results. Although the works dealing with this topic are scarce –and even more considering broad geographical scales–, Perveen et al. (2019), working with a very limited number of samples per land use, also found no differences in the PE among cropland types.

4.2. Cropland controls PE via soil microbes and C content

The agricultural use of soils indirectly influences PE via changes in SOC content, basal respiration and MBC. Additionally, SEM indicated that land use (as a categorical variable) exerts a direct influence on priming effect. This direct effect may be accounting for other factors that inherently change between soils under contrasting land uses and that were not considered in the analytical characterization of soils used in the present work; for example, SOM structure and/or composition (Mueller and Koegel-Knabner, 2009; Panettieri et al., 2020). Cropland soils, with less SOC, MBC and respiration (in comparison to seminatural soils), are more C limited and rapidly respond to fresh C inputs resulting in higher positive feedbacks of CO_2 to the atmosphere compared with seminatural soils (Bastida et al., 2019). Thus, given the low SOC content in croplands, microorganisms inhabiting these soils can be more responsive to the added glucose -specially r-strategists-, increasing their activity and biomass quickly with a further accelerated SOC mineralization. The SOC mineralization would go in parallel with microbial community changes towards more K-dominated strategists (Fontaine et al., 2003). This dynamic would explain the less negative PE in cropland soils compared to seminatural ones.

Some agricultural practices associated with land-use intensification (e.g., excessive tillage) strongly reduce SOC content (Wei et al., 2014; Bouchoms et al., 2017), while other practices related to the application of organic amendments can increase SOC content and decrease soil pH (Delgado-Baquerizo et al., 2017; Malik et al., 2018; Zamanian et al., 2018). Modelling approaches highlight that the higher SOC contents and the lower pH increased basal respiration, MBC content and extracellular enzyme activities (β-glucosidase and cellulase activities) and decreased the PE in European seminatural soils. Therefore, such practices (e.g., application of organic amendments, cover crops, etc.) can play a key role in regulating the PE and reducing CO_2 emissions from soil. Considering the increasing interest in moving towards a greener agriculture (post-2020 Common Agricultural Policy - European-Commission (2018)), our large-scale findings offer further evidence on the soil management practices that better align with European Union strategies for soil C sequestration and climate change mitigation.

5. CONCLUSIONS

To the best of our knowledge, this is the first study on the impact of land use on the PE at European scale. The PE in cropland and seminatural soils (under forests and grasslands) were on average negative, meaning that microorganisms preferentially changed from SOC decomposition to glucose mineralization as a consequence of limitation by available C. Cropland soils showed less negative PE than seminatural soils as microorganisms in these ecosystems are more strongly C limited and respond intensively to fresh C inputs. The PE were similar among the three studied crop types: cereals, non-permanent industrial crops (sunflowers and rape) and woody orchards, which points out that crop type is not a driving factor of PE in croplands. Further studies are needed in this regard in order to identify potential drivers of PE in cropland soils subjected to different agricultural practices. The modelling approaches did demonstrate that soil agricultural use -in comparison with forests and grasslands- has a key role in shaping the PE through changes of basal respiration, MBC and SOC. This demonstrates that priming effect -despite being highly methodologically challenging- can be successfully modelled at continental scale, which has important implications to generate more accurate global C cycling models and to develop more sustainable agroecosystems. Despite the advance that the present work represents to the field of soil PE, it is important to recognize that the conclusions of this study were drawn considering the addition of only one dose of glucose as well as a sole and constant temperature and soil water content, which reflects potential rather than actual PE. Considering large differences in the N stocks and C/N ratios between ecosystems and land use types, we assume that the next step in the evaluation and understanding of PE on large scale should be the effects of N loading (or fertilization for croplands) on acceleration of SOC turnover. From this point of view, it is also crucial to assess the release or immobilization of N and P within induced PE. The ecosystems with common positive PE should release nutrients stored in soil organic matter. This could be a useful strategy for mobilization of some nutrients (e.g., P) that are stored in cropland soils in high amounts but remain unavailable for plants until microbial enzymes release them in mineral form.

CONFLICTS OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article (Table S1).

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Figure captions

Fig. 1. Box plots comparing soil organic carbon (a) and microbial biomass carbon (b) contents, pH (c) and bacterial (d) and fungal (e) abundances between cropland and seminatural (forest and grasslands) soils. Different letters above each bar denote significance of differences at $p<0.05$ (PERMANOVA). 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.

Fig. 2. Box plots comparing basal respiration (a, c) and priming effects (b, d) between cropland and seminatural (forest and grasslands) soils (a, b) and among crop types (c, d; CER, cereals; OIL, non-permanent industrial crops; ORC, orchards) after 35 days of incubation. Different letters above each bar denote significance of differences at $p<0.05$ (PERMANOVA). 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.

Fig. 3. (a) Random Forest mean predictor importance (% increase in MSE -mean square error-) of the selected variables as predictors of the priming effects (PE) in cropland and seminatural (grasslands and forests) soils across Europe (n=126). (b) Standardized total effects (STE) of the selected factors on the PE derived from the Structural Equation Model (SEM). This is the sum of direct and indirect effects of each predictor on PE. For (b) and (c), significance levels of each predictor are shown at *$p<0.05$ and **$p<0.001$ and predictors belonging to the same environmental category were represented with the same color according to the legend in (a) (c) 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. Only significant effects ($p<0.05$) are indicated, and significance levels are shown at *$p<0.05$ and ** $p\leq 0.001$. R^2 denotes the proportion of variance explained. The model was satisfactorily fitted to data, as suggested by non-significant $\square^2$ values, non-parametric bootstrap and by values of RMSEA. MBC = Microbial biomass carbon. LU = Land use. MAT = Mean annual temperature. NPP = Net primary production. AI= aridity index. Sand = sand content (>0.063, <2.0 mm). SOC= Soil organic carbon. MBC= Microbial biomass carbon. Bacteria = FAME-based bacterial abundance. Fungi = FAME-based fungal abundance. P and K = Total phosphorus and potassium contents, respectively.

Fig. 4. Selected dependences of priming effects on microbial parameters evaluated by regression analyses. (a) Priming effect in cropland and seminatural (forest and grassland) soils vs. basal microbial respiration. (b) Priming effect vs. microbial biomass carbon. (c) Priming effect vs. soil organic carbon. All regressions are significant at $p<0.001$. Shaded areas represent 95% confidence interval for the regression line.

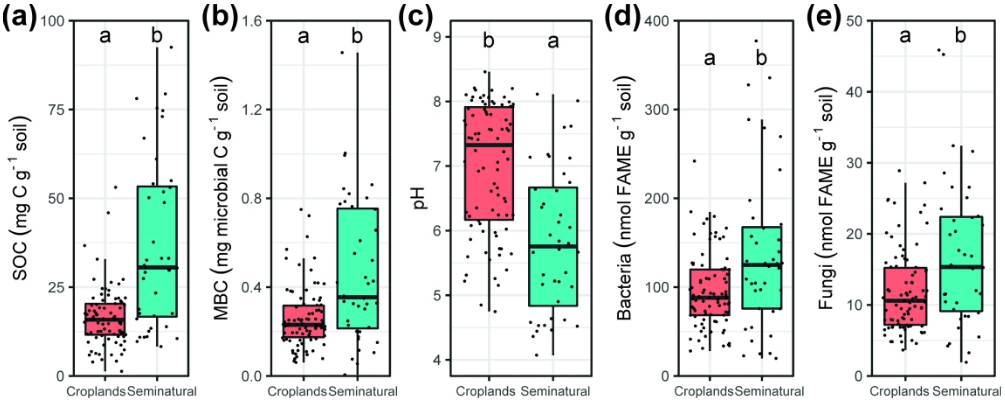

Fig. 1. Box plots comparing soil organic carbon (a) and microbial biomass carbon (b) contents, pH (c) and bacterial (d) and fungal (e) abundances between cropland and seminatural (forest and grasslands) soils. Different letters above each bar denote significance of differences at $p<0.05$ (PERMANOVA). 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.

189x75mm (600 x 600 DPI)

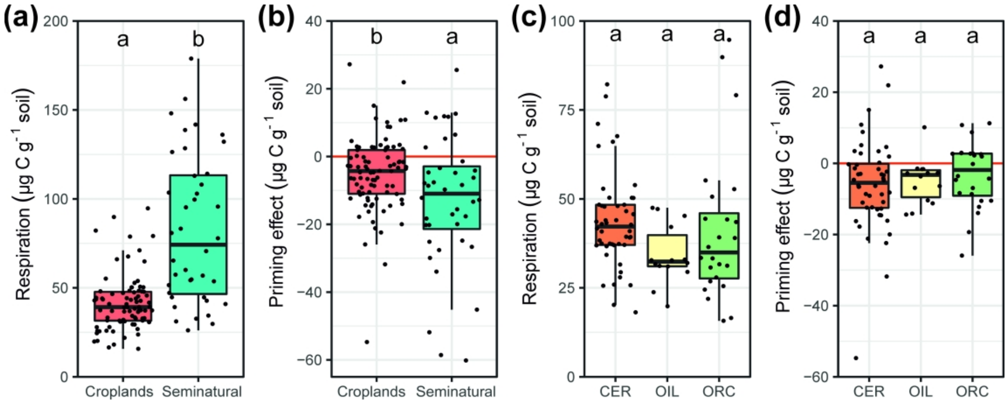

Fig. 2. Box plots comparing basal respiration (a, c) and priming effects (b, d) between cropland and seminatural (forest and grasslands) soils (a, b) and among crop types (c, d; CER, cereals; OIL, non-permanent industrial crops; ORC, orchards) after 35 days of incubation. Different letters above each bar denote significance of differences at $p<0.05$ (PERMANOVA). 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.

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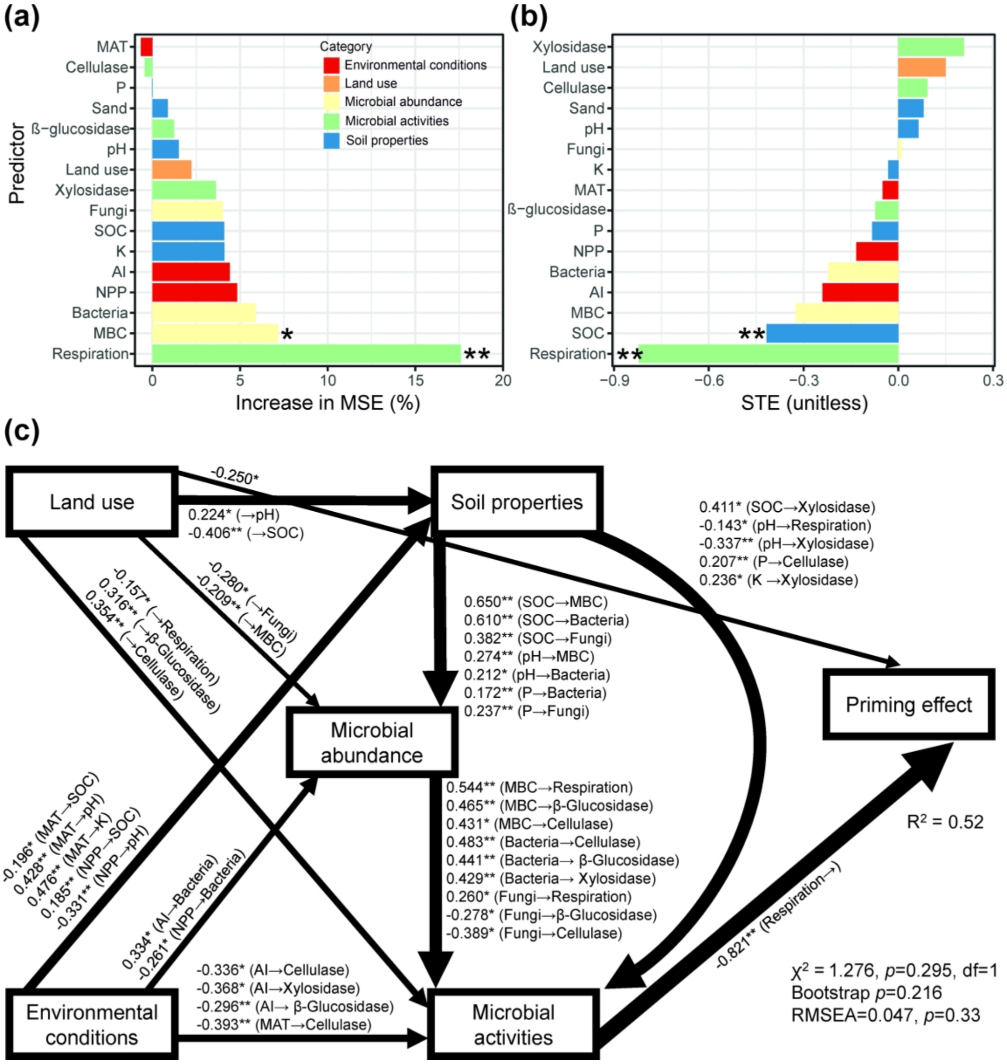

Fig. 3. (a) Random Forest mean predictor importance (% increase in MSE -mean square error-) of the selected variables as predictors of the priming effects (PE) in cropland and seminatural (grasslands and forests) soils across Europe (n=126). (b) Standardized total effects (STE) of the selected factors on the PE derived from the Structural Equation Model (SEM). This is the sum of direct and indirect effects of each predictor on PE. For (b) and (c), significance levels of each predictor are shown at *p<0.05 and **p<0.001 and predictors belonging to the same environmental category were represented with the same color according to the legend in (a) (c) 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. Only significant effects (p<0.05) are indicated, and significance levels are shown at *p<0.05 and ** p≤0.001. R2 denotes the proportion of variance explained. The model was satisfactorily fitted to data, as suggested by non-significant ☐2 values, non-parametric bootstrap and by values of RMSEA. MBC = Microbial biomass carbon. LU = Land use. MAT = Mean annual temperature. NPP = Net primary production. AI= aridity index. Sand = sand content (>0.063, <2.0 mm). SOC= Soil organic carbon. MBC= Microbial biomass carbon. Bacteria = FAME-based bacterial abundance. Fungi = FAME-based fungal abundance. P and K = Total phosphorus and potassium contents, respectively.

189x200mm (300 x 300 DPI)

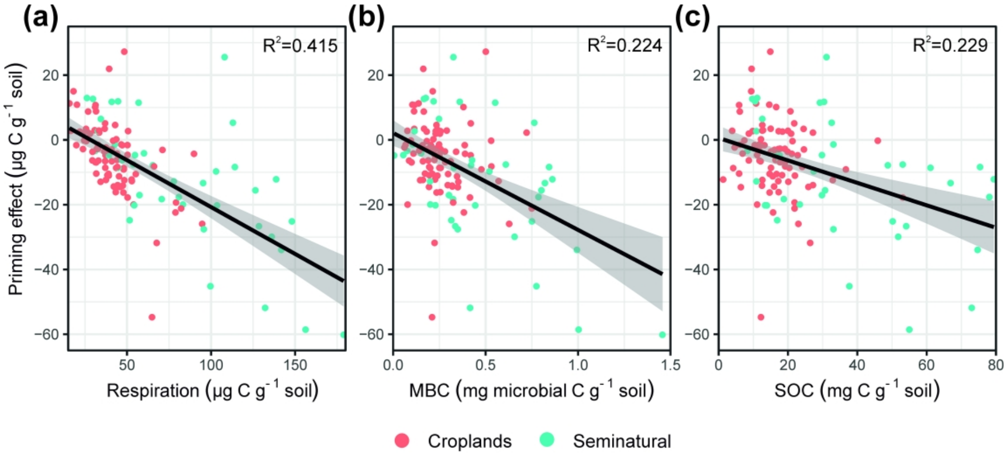

Fig. 4. Selected dependences of priming effects on microbial parameters evaluated by regression analyses. (a) Priming effect in cropland and seminatural (forest and grassland) soils vs. basal microbial respiration. (b) Priming effect vs. microbial biomass carbon. (c) Priming effect vs. soil organic carbon. All regressions are significant at p<0.001. Shaded areas represent 95% confidence interval for the regression line.

189x85mm (600 x 600 DPI)