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Short communication

Priming effect increases with depth in a boreal forest soil

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ABSTRACT

Climate warming increases labile carbon (C) inputs to soil through increased photosynthesis and C allocation belowground. This could counterintuitively lead to losses of soil C via priming effects (PE): the stimulation of soil organic matter (SOM) decomposition caused by labile C addition. Systematic quantification of PEs in different ecosystems is needed. We measured PEs of free-living soil microbes in different layers of a boreal forest soil, and found that the relative magnitude of the PE increased with soil depth. The relationship between relative PE and the added glucose amount also depended on the soil layer. Our results indicate that the decomposition of SOM in deeper soil layers could be significantly increased due to PE, if labile C inputs into these layers increase.

Increased root exudation (Yin et al., 2013), and increased fine-root biomass especially in deeper soil layers (Norby et al., 2004; Leppälammi-Kujansuu et al., 2013, 2014) have been observed in response to warming and elevated CO_2 concentrations in field experiments. This could lead to increased SOM decomposition through PEs (Fontaine et al., 2004, 2007). The dependence of PE on the amount of added C and on the soil properties needs to be quantified before these processes can be explicitly represented in soil C models (Paterson and Sim, 2013). Even though positive PEs have been suggested to be especially important in N-limited ecosystems (Dijkstra et al., 2013), PE studies in boreal ecosystems are scarce (Chigineva et al., 2009; Fan et al., 2013; Lindén et al., 2014; Linkosalmi et al., 2015). This gap of knowledge is particularly critical given that boreal regions contain 30% of all terrestrial C, and boreal forest soils store 625 Pg of C, twice as much as temperate and tropical forest soils together (Kasischke, 2000).

We sampled four layers of a boreal (Norway spruce) forest soil (haplic podzol) in October 2013: the organic layer (O), and three mineral soil layers (E, B1, B2, Table 1). For more details on the study site and soil characteristics, see Karhu et al. (2010a, 2010b). Contrasting properties of these layers allowed us to test the hypothesis that relative C and N availability determine the PE (e.g. Kuzyakov, 2002; Chen et al., 2014). The soils were sieved to 2 mm (mineral soil layers) or 4 mm (organic layer), and let to recover from the disturbance of sampling and sieving for ca. two weeks at 6 °C before starting the incubations. Soil microbial biomass (C_{mic}) was determined using the chloroform fumigation extraction (CFE) method (Vance et al., 1987; as modified in Blagodatskaya et al., 2011), except that no correction factor (k_{EC}) was used (as in Leckie et al., 2004) (Table 1) since this factor likely varies between the soil layers. Soil dry weight (d.w.) and water holding capacity (WHC) were determined as in Karhu et al. (2014). We had four analytical replicates in the laboratory incubations, but no field replication, because we wanted to study the process of priming effect in different soil layers, with no intention to predict C losses on an areal basis.

Glucose was used as a model compound to study rhizosphere PE (Derrien et al., 2004). ^{13}C labelled glucose (≈2 atom %) was added to soil at different amounts relative to CFE biomass size (0, 0.125, 0.25, 0.5, 1 and 2 times C_{mic}, Table 1). This led to different amounts of glucose added per g soil in the different soil layers, as C_{mic} also decreased with depth (Table 1). We chose to add glucose relative to biomass size (not relative to soil dry weight) as Blagodatskaya and

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Table 1
Initial soil properties in soil layers and glucose additions in the different treatments. Control treatment received H_2O only (glucose addition 0 × C_{mic}).

Soil layer	Depth	C	N	C:N	Dissolved N	N_{mic}	DOC	C_{mic}	Glucose addition (mg glucose C g^{-1} soil d.w.)				
	(cm)[a]	(%)	(%)	Ratio	(μg N g^{-1} soil)		(mg C g^{-1} soil)		0.125 × C_{mic}	0.25 × C_{mic}	0.5 × C_{mic}	1 × C_{mic}	2 × C_{mic}
O	0–7	28.7	0.97	29.7	79.3	195	0.93	2.90	0.36	0.72	1.45	2.90	5.80
E	7–17	0.59	0.06	9.3	5.73	4.02	0.05	0.13	0.02	0.03	0.06	0.13	0.25
B1	17–32	1.57	0.09	17.8	6.96	3.42	0.11	0.11	0.01	0.03	0.06	0.11	0.22
B2	32–47	0.4	0.05	8.8	4.28	0	0.05	0.05	0.006	0.01	0.02	0.05	0.09

- C and N % values represent total organic carbon and total N in each layer (measured using varioMax CN analyser, Germany). Dissolved N and DOC are the total N (including dissolved organic and inorganic N) and organic C contents of K_2SO_4-extracts of non-fumigated control samples, respectively. C_{mic} and N_{mic} are the C and N contents released by fumigation (fumigated-control samples), measured using Shimadzu TOC-V cph/cpn analyzer (Kyoto, Japan).
[a] Below soil surface.

Kuzyakov (2008) have stressed the need to perform substrate additions relative to biomass size in PE studies. According to the authors, the amount of added available substrate in relation to the microbial C is a key factor affecting the direction and type of the priming effect. Glucose was added in solution with enough water to raise each soil to 60% of WHC. Control samples received deionized water only.

After glucose addition, the soils (12–35 g depending on layer) were placed inside 500 ml glass bottles, which were closed and flushed with CO_2-free air, and then incubated at 14 °C for 11 days. During the incubation, CO_2 concentrations inside the bottles were measured daily or every other day using gas chromatography (Hewlett Packard 6890). For determining the ^{13}C contents of produced CO_2 at the end of incubation, a 20 ml gas sample was taken from each bottle by syringe, injected into a pre-evacuated 12 ml Exetainer vial (Labco limited, Lampeter, UK), and analyzed using an isotope ratio mass spectrometer (Thermo Finnigan GasBench II connected to Thermo Finnigan Delta plus Advantage). For organic layers, this sampling was repeated four times during the incubation (2, 5, 9 and 11 days after labelling) to avoid CO_2 concentrations exceeding 20 000 ppm. After each gas collection, the bottles were flushed with CO_2 -free air. Results are presented for the cumulative sum on day 11. Following Sturm et al. (2015) we measured and, using mass balance calculations, corrected our ^{13}C results for the contamination due to residual CO_2 remaining inside the Labco pre-evacuated Exetainers (more information in Supplementary materials). The effects of this contamination on our data were minimal for two reasons: 1) we collected high concentrations of CO_2 at the end of our experiment, and 2) used highly labelled ^{13}C-glucose (see below), and thus it did not affect the trends observed with priming effect (see discussion) and interpretation of our results (see Supplementary information).

The amount of CO_2 (μg CO_2–C g $soil^{-1}$) derived from added glucose ($C_{glucose}$) was calculated based on the total CO_2–C amount (C_{tot}) and its ^{13}C content (at% sample) at the end of the incubation:

$$C_{glucose} = C_{tot}\frac{at\%sample - at\%control}{at\%glucose - at\%control} \quad (1)$$

where at% glucose is the atom% ^{13}C of the added glucose (2.04 at%) and at% control is the atom% ^{13}C of control soil-derived CO_2 (varying between 1.07 and 1.08 at% depending on soil layer). The amount of CO_2 originating from the decomposition of soil organic matter (SOM) was calculated as C_{SOM}=C_{tot}-$C_{glucose}$. PE was then calculated as C_{SOM}-$C_{control}$, where ($C_{control}$) is the total CO_2–C produced by control samples receiving water only. Standard errors were calculated according to Kuzyakov and Bol (2004). Regression analysis was used to relate the magnitude of PE (at the highest glucose addition of 2 times C_{mic}) to soil properties. Linear regressions were fitted using IBM SPSS statistics 22.

Our results show that the relative magnitude of PE (% increase in SOM decomposition) increased with soil depth (Fig. 1), consistent with the findings of Fontaine et al. (2007) and Hartley et al. (2010). When plotted against the amount of added glucose, the relative PE magnitude formed a saturating curve for layers B1 and B2 (as in Guenet et al., 2010; Paterson and Sim, 2013). In the E and O layers, relative PE was small regardless of the added glucose amount (Fig. 1). Absolute PE was highest in layer B1 (Fig. 2).

The relative PE magnitude within the soil profile was negatively related to C availability (determined as cumulative CO_2–C produced per g soil C during the incubation by the control samples) ($R^2 = 0.96$, $p = 0.02$). Secondary explanatory factors were related to N availability, but the positive relationship with C:N ratio of the "active SOM pool" (Kuzyakov et al., 2000; 2002), defined here as the sum of microbial and dissolved SOM pools (i.e. C:N ratio of the flush released by fumigation-extraction) and the negative relationship with % of total soil N in the active pool, were not statistically significant ($R^2 = 0.73$, and $R^2 = 0.76$, respectively) and these correlations would have to be confirmed in later studies with a larger number of soils. There is a possibility that these relationships are not causal ones as also other factors vary among the soil layers (e.g. soil microbial communities).

However, greater PE magnitude in deeper soil layers supports both the idea of energy or labile C availability limiting SOM decomposition in deeper soil layers (Fontaine et al., 2004, 2007; Paterson and Sim, 2013), and secondarily that PE is more

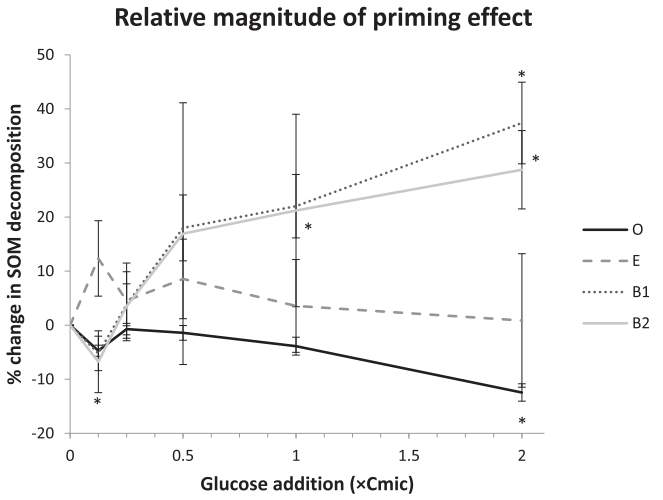

Fig. 1. Relative magnitude of PE in different soil layers (**mean ± S.E., n = 4**). Statistically significant PEs, i.e. with the 95% confidence intervals not overlapping 0 are marked with an asterisk. The relative PE magnitude was small regardless of the added glucose amount in the O and E layers, while it increased sharply with added glucose amount in layers B1 and B2. At glucose additions >0.5 × C_{mic}, the PE started to level off in layer B2, but was still linearly increasing in layer B1, albeit at a slower rate than at lower concentrations.