Soil Biology & Biochemistry 65 (2013) 254–263

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Microbial gross organic phosphorus mineralization can be stimulated by root exudates – A ^{33}P isotopic dilution study

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ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form
27 May 2013
Accepted 30 May 2013
Available online 19 June 2013

Keywords:
Gross organic P mineralization
^{33}P isotopic dilution
Microbial activity
Rhizosphere
Root exudates
Phosphatase
Priming effects
Stoichiometry
Rhizodeposition

ABSTRACT

Phosphorus (P) is one of the most important nutrients for plant growth. While most studies on microbial P mobilization, i.e. on mineralization of organic P and solubilization of inorganic P, focus on mycorrhiza, P mobilization by non-mycorrhizal microorganisms in soil is little explored. In this study we address the question whether root exudates stimulate organic P mineralization by non-mycorrhizal microorganisms. A ^{33}P isotopic dilution approach was applied to investigate microbial gross P mineralization in top- and subsoil horizons of three forest soils differing in P concentrations (Leptosol, Podzol, and Cambisol). To simulate the effects of root exudates on microbial gross P mineralization, glucose, alanine, and methionine were added in rhizosphere-relevant concentrations (12 mg carbon (C) g^{-1} soil organic C). Based on ^{33}P isotopic dilution we showed that glucose and alanine addition increased gross P mineralization rates up to a factor of 20 and 31, respectively. In contrast, methionine had little effect on microbial gross P mineralization rates. Phosphatase activity was increased most strongly due to the addition of alanine and glucose by factors of up to 6 and 4, respectively. Fifteen days after addition of artificial root exudates, microbial P concentrations and ^{33}P recovery in the microbial biomass were only slightly and not consistently changed. In conclusion, the results show that alanine and glucose can stimulate microbial gross P mineralization and phosphatase activity, and that structure and stoichiometry of root exudates significantly shape the extent of stimulation of microorganisms. Our study indicates that stimulation of non-mycorrhizal microorganisms by root exudates might be an important strategy of plants to increase the availability of P in soils.

1. Introduction

Decreasing resources of rock phosphate suitable for fertilizer production (Cordell et al., 2009) have recently triggered interest in mineralization of organic phosphorus (P) already present in soil. P is one of the most important limiting nutrients for plant growth. Many agricultural systems are strongly dependent on continuous P inputs by fertilization. In contrast, biota in forest ecosystems has developed highly efficient strategies for mobilization, uptake, recycling and usage of P (Wardle et al., 2004). Therefore, forest soils represent suitable systems to study microbial P mobilization, i.e. solubilization of inorganic P (P_i) and mineralization of organic P (P_o).

The chemical forms of P in soils differ not only with parent material, soil pH and vegetation cover, but also with time and the extent of pedogenesis (Walker and Syers, 1976). The organic P pool increases with soil development, but tends to decline again in highly weathered soils. Calcium-phosphates represent the primary mineral source of P_i in only moderately weathered soils with neutral to alkaline pH, whereas iron (Fe) and aluminum (Al) phosphates and P_i bound or occluded by Fe and Al (hydr)oxides predominate in acidic and more progressively weathered soils (Walker and Syers, 1976). Due to rapid precipitation because of low solubility products of P-containing minerals and high affinity to positively charged surfaces (namely pedogenic oxides) only a small percentage of P present in soil is soluble, and thus plant available (Attiwill and Adams, 1993).

Plants, fungi, and bacteria have developed several mechanisms to mobilize P. Among the most investigated are the release of protons that lead to a decrease in soil pH, the release of organic and inorganic ligands and chelators such as bicarbonate or carboxylic anions, and the release of phosphatases that mineralize P_o (Uroz et al., 2009; Jones and Oburger, 2011). While chemical solubilization of P_i by plants and mycorrhiza has been studied in detail (Hinsinger, 2001), mobilization of P by non-mycorrhizal

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0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.05.028

microorganisms has received less attention. Achat et al. (2010, 2012) reported that microbial net organic P mineralization was correlated with organic carbon (C) mineralization and soil C/P ratio in temperate Podzols under forest. In agreement with this result, Spohn and Kuzyakov (2013) found in an incubation experiment with ^{14}C and ^{33}P labeled glucose-phosphate that microbial organic P mineralization in some temperate forest soils was driven by the microbial need for C.

Microorganisms in soils are usually C limited and therefore remain in a dormant state. However, trace amounts of easily degradable organic C can strongly stimulate their activity (Joergensen et al., 1990; De Nobili et al., 2001). Root exudates that are composed of low molecular weight organic substances (LMWOS) represent a significant source of easily degradable organic C (Kuzyakov and Domanski, 2000). Small additions of LMWOS such as amino acids or monosaccharides to soil strongly increase organic C mineralization (Blagodatskaya et al., 2009; Dorodnikov et al., 2009). Especially glucose, which serves as an energy source to microorganisms, has a strong effect on microbial activity. In contrast, amino acids that are mainly used as nitrogen (N) and – less importantly – as C source by microorganisms have a smaller effect on organic C mineralization (Marstorp, 1996; Hamer and Marschner, 2004).

It has been stated frequently that inputs of organic carbon (OC) by roots into soils strongly shape terrestrial nutrient cycling (Högberg and Read, 2006; Kuzyakov and Domanski, 2000). However, studies on the effects of root exudates on nutrient mobilization are scarce. While few studies explore impacts of rhizodeposits on organic N mineralization (Norton and Firestone, 1996), nitrification, and denitrification (Priha et al., 1999a,b), there is – to our knowledge – no study on impacts of root exudates on gross P mineralization (see also Richardson et al., 2009). So far, effects of plants on microbial P mineralization have only been estimated by phosphatase activity with contrasting results. Rhizodeposition increased phosphatase activity in a girdling experiment with beech trees (Koranda et al., 2011). In contrast, in a girdling experiment with conifers in a subalpine forest phosphatase activity was not affected by rhizodeposition (Weintraub et al., 2007).

The objective of this study was to test the effect of LMWOS on phosphatase activity, P mineralization, and microbial P immobilization. We hypothesized, first, that LMWOS stimulate phosphatase activity, and P mineralization; and second, that the extent of the stimulation depends on the stoichiometry of the LMWOS. To test these hypotheses we determined gross organic P mineralization by ^{33}P dilution in soils treated with single LMWOS that form an important part of root exudates. To gain insight into potential P mineralization we also determined phosphatase activity in the soils. Finally, we measured ^{33}P uptake and microbial P concentrations to estimate microbial P immobilization. Since P mineralization rates are assumed to differ strongly among soils depending on their P status and forms, three soils, a Leptosol, a Podzol, and a Cambisol were studied. Single substances have frequently been applied to soil to study the effects of root exudates (Hamer and Marschner, 2004; Blagodatskaya et al., 2009). We used three LMWOS; alanine, methionine, and glucose. Glucose was chosen as the most abundant plant monosaccharide (Chesire, 1979; Derrien et al., 2004) and important energy source for microorganisms (Blagodatskaya et al., 2009). Amino acids were chosen since they are an important constituent of root exudates (Fischer et al., 2007) providing N to soil microorganisms. More specifically, we selected the amino acids alanine and methionine because the latter, in contrast to alanine, contains sulfur (S).

2. Material and methods

2.1. Soils

Soil was sampled from sites located in central Germany in forests around the city of Göttingen. The mean annual temperature of the study area is 8.7 °C and the mean annual precipitation is approximately 640 mm. The Leptosol is located in the east of Göttingen (51°33′23 N, 9°58′25 E) in the Göttinger Wald. The soils in Göttinger Wald are largely developed from shell limestone and a mixed deciduous forest can be found featuring *Fagus sylvatica*, *Acer* ssp., and *Sorbus aucuparia*. The Podzol and the Cambisol are located in the Bramwald in the southwest of Göttingen. The Podzol (51°31′01 N, 9°39′15 E) was formed from tertiary sands, while the Cambisol (51°30′51 N, 9°39′08 E) has developed from basalt. The vegetation on the Podzol is strongly dominated by *Picea abies*, while *F. sylvatica* is the dominant species on the Cambisol. The soils differ strongly in texture, pH, C/N ratios, and P_i concentrations (Table 1). The Podzol exhibits the highest C/N ratio and the lowest P concentrations. The Leptosol and the Cambisol show similar C/N ratios, but the Leptosol contains larger P_i concentrations than the Cambisol (Table 1).

2.2. Soil sampling and preparation

Soils were sampled in November 2011. Three profiles were dug at each soil type. We sampled the topsoil horizon at a depth of 1–6 cm, and a subsoil horizon of the Leptosol and of the Podzol at a depth of 21–25 cm. One sample per horizon was taken from each profile. The soils were sieved (2 mm) and pre-incubated at 20 °C and 40% water holding capacity for six weeks prior to the incubation experiments in order to let them reach equilibrium under laboratory conditions. This pre-incubation has been found to be crucial for ^{33}P isotopic dilution experiments (Bünemann et al., 2007).

2.3. ^{33}P isotopic dilution

Gross P mineralization rates were determined by isotopic dilution of ^{33}P. This method is based on a decrease in ^{33}P specific activity

Table 1
Texture, pH, organic carbon (OC), nitrogen (N), C/N, total phosphorus (P), and microbial carbon (C_{mic}) in the topsoil horizon (1–6 cm) and a subsoil horizon (21–25 cm) of Leptosol, Podzol, and Cambisol. The values depict means that were calculated from the independent analyses of three soil profiles per soil type. Values in brackets depict standard deviations.

Soil type	Horizon	Texture [%]			pH_{H_2O}	OC [g kg^{-1}]	N [g kg^{-1}]	C/N[a]	P [g kg^{-1}]	C_{mic}[b] [g kg^{-1}]
		Sand	Silt	Clay						
Leptosol	Topsoil [Ah]	1.3 (±0.2)	57.5 (±2.8)	41.3 (±2.9)	5.6 (±0.0)	81.1 (±6.5)	4.7 (±0.4)	17.2 (±1.9)	0.54 (±0.01)	1.05 (±0.01)
Leptosol	Subsoil [Bw]	1.1 (±0.1)	53.9 (±1.9)	45.0 (±1.9)	4.8 (±0.0)	15.6 (±3.9)	1.2 (±0.1)	12.6 (±1.0)	0.32 (±0.01)	0.25 (±0.01)
Podzol	Topsoil [Ahe]	69.2 (±5.9)	26.0 (±4.2)	4.8 (±1.8)	4.0 (±0.1)	50.0 (±12.6)	2.3 (±0.4)	21.2 (±1.0)	0.07 (±0.02)	0.27 (±0.01
Podzol	Subsoil [Bhs]	65.0 (±1.3)	22.4 (±5.2)	12.6 (±6.1)	4.0 (±0.0)	28.1 (±3.5)	0.9 (±0.2)	31.6 (±2.0)	0.13 (±0.01)	0.15 (±0.01)
Cambisol	Topsoil [Ah]	22.6 (±1.4)	55.9 (±1.6)	21.5 (±1.2)	5.0 (±0.0)	60.0 (±4.1)	3.1 (±0.2)	19.3 (±0.8)	0.38 (±0.03)	0.57 (±0.01)

[a] Total OC and N were determined with an element analyzer (Vario EL, Elementar).
[b] C_{mic} was determined by fumigation-extraction and the samples were measured with a TOC analyzer (Dohrmann), no conversion factor was used.

(i.e. ^{33}P activity divided by the concentration of inorganic P) in the soil solution due to a dilution of $^{33}P{-}PO_4^{3-}$ with soil-derived $^{31}P{-}PO_4^{3-}$. This dilution of $^{33}P{-}PO_4^{3-}$ is driven by physicochemical and by biochemical processes such as the mineralization of organic P (Oehl et al., 2001; Frossard et al., 2011). The ^{33}P dilution that is exclusively driven by physicochemical processes can be modeled based on a short-term (100 min) batch experiment. The biochemically driven gross P mineralization can be calculated by subtracting this modeled ^{33}P dilution from the measured ^{33}P dilution. An incubation experiment was conducted to determine ^{33}P dilution during 14 days. The incubation experiment consisted of 72 experimental units: Five soils (three topsoils and two subsoils), four treatments, and three replicates. The treatments consisted of one single addition of alanine, methionine or glucose, and no addition of LMWOS (control). 10 g dry mass equivalent of moist soil were equilibrated with 95 ml distilled H_2O in 200 ml plastic vials on a temperature-regulated shaker (Gallenkamp) at 20 °C and 120 rpm in the dark. After 18 h, alanine, methionine or glucose dissolved in distilled H_2O was added and the volume was adjusted to 99 ml. The control was adjusted to 99 ml without any further additions. The amount of alanine, methionine and glucose added was 12 mg C g^{-1} SOC. This amount of OC addition was chosen since it represents a high, but still realistic flux of C that soils might receive from roots during approximately 10 days by exudation (Kuzyakov and Domanski, 2000).

The experiment was started directly after the addition of the LMWOS by adding 34 kBq of $^{33}P{-}PO_4^{3-}$ in 1 ml of distilled H_2O to all samples and by returning the samples to the shaker. The incubation conditions were maintained as during the pre-incubation throughout the experiment, i.e. 120 rpm and 20 °C. Samples were taken 1.6, 2.2, and 6 h, and 1, 3, 5, 7, 10, and 14 days after the beginning of the experiment. For this purpose 1.5 ml were removed from the soil suspension with a pipette (and a pipette tip from which we cut the tip in order to facilitate the uptake of the soil suspension) and immediately centrifuged at 20 000 × *G* and 4 °C for 2 min (Centrifuge 5417R, Eppendorf). The supernatant was removed, transferred to a new vial and again centrifuged at 20 000 × *G* and 4 °C for 3 min. The soil solution was centrifuged because filtration of the clayey Leptosol was time consuming. We found that the centrifugation of the soil solution and filtration through filters with a pore size of 0.20 μm result in nearly identical ^{33}P activity in the soil solution (Appendix 1). ^{33}P activity was measured with the scintillation cocktail Rotiszint Ecoplus (Roth) using a multi-purpose scintillation counter (Beckman–Coulter). Samples were stored at −14 °C, and finally P_i in the soil solution of all samples was determined colorimetrically with malachite green on a multilabel counter (Victor^3, Perkin Elmer). The recovery of ^{33}P in soil solution was calculated as the percentage of the initially added ^{33}P activity.

2.4. ^{33}P isotopic dilution during 100 min

A 100-min experiment was conducted to determine the ^{33}P dilution, which is considered to be solely due to physicochemical mechanisms (Oehl et al., 2001; Bünemann et al., 2007; Frossard et al., 2011). The experiment was conducted in the same way as the 14-day ^{33}P dilution experiment (see 2.3). Samples were taken after 1, 10, 30, 70, and 100 min with a pipette and analyzed for ^{33}P and P_i as described above. We did not use a microbial inhibitor, since we observed that the addition of $HgCl_2$ – an inhibitor commonly used in soil biology – changed soil properties as indicated by flocculation and increased sedimentation rates of soils in water.

2.5. ^{33}P dilution due to biotic activity

The results of the short-term ^{33}P dilution experiment were used to model the part of ^{33}P dilution that is driven by physicochemical processes during 14 days. The proportion ($r(t)/R$) of the initially introduced activity (R) found in soil solution at any time (t) up to 14 days was calculated according to Fardeau et al. (1985) as

$$\frac{r(t)}{R} = \left(\frac{r_{1\ \mathrm{min}}}{R}\right)\left(t + \left(\frac{r_{1\ \mathrm{min}}}{R}\right)^{1/n}\right)^{-n} + \frac{r_{\mathrm{inf}}}{R}$$

where $r_{1\ \mathrm{min}}/R$ and n are parameters derived from the 100-min experiment. The parameter $r_{1\ \mathrm{min}}/R$ is the proportion of the initial ^{33}P activity remaining in solution after one minute, and n is the slope of the regression of $\log[r(t)/R]$ and $\log[t]$. The ratio r_{inf}/R is the maximum possible dilution of the isotope, calculated by the ratio 10^*C_p/total inorganic P, with C_p being the P_i concentration in the soil solution (see Table 2).

The amount of the isotopically exchanged P at any time t ($E(t)$) was calculated according to Oehl et al. (2001) and Bünemann et al. (2007) as follows:

$$E(t) = \frac{C_p}{\frac{r(t)}{R}}$$

The modeled $E(t)$ ($E(t)_{\mathrm{mod}}$) was calculated from the modeled $r(t)/R$ with the C_p concentrations of the 100-min experiment according to Oehl et al. (2001). The difference between the measured $E(t)$ ($E(t)_{\mathrm{mes}}$) and the modeled $E(t)$ ($E(t)_{\mathrm{mod}}$) is assumed to be the amount of P that is biochemically mineralized (Oehl et al., 2001):

$$\Delta E(t) = E(t)_{\mathrm{mes}} - E(t)_{\mathrm{mod}}$$

By dividing the amount of the biochemically mineralized P at any time t by the duration of incubation (Δt), the gross P mineralization rate ($P_{\mathrm{mineralization}}$) is obtained according to Oehl et al. (2001):

$$P_{\mathrm{mineralization}} = \frac{\Delta E(t)}{\Delta t}$$

2.6. Total phosphorus and ^{33}P in the microbial biomass

After the incubation experiment, total P and ^{33}P in the microbial biomass were determined according to Brookes et al. (1982). The soils of the incubation experiment were centrifuged for 15 min at 4000 × *G* (6K15, Sigma), and allowed to dry for 18 h at 25 °C. Briefly, 5 g soil were fumigated with chloroform for 24 h before extraction with 100 ml 0.5 M $NaHCO_3$. As a control, another 5 g soil were directly extracted with 100 ml 0.5 M $NaHCO_3$. Total P in both extracts was measured by ICP-AES (Spectroflame, Spectro), ^{33}P activity in the extracts was measured using a multi-purpose scintillation counter (Beckman–Coulter), and the ^{33}P activity in the microbial biomass was calculated as the difference between the activity of the fumigated sample and the non-fumigated control. Microbial P (P_{mic}) is given as the difference of the P concentration of the extract of the fumigated sample and the P concentration of the extract of the non-fumigated control. We did not correct for sorption of inorganic P. We also did not use a conversion factor as suggested by Oberson and Joner (2005). This probably leads to a slight underestimation of the microbial P, while it avoids the uncertainty associated with the conversion factor.

2.7. Phosphatase activity

Phosphatase activity was determined in a 10-day incubation experiment with the fluorogenic substrate 4-methylumbelliferyl-phosphate according to Marx et al. (2001) with modifications by German et al. (2011). The incubation experiment consisted of 60 experimental units: five soils (three topsoils and two subsoils),

Table 2
Parameters of isotopic exchange derived from the 100-min incubation ($r_{1\ min}/R$, n, C_p) as well as the concentrations of isotopically exchanged P after one minute ($E_{1\ min}$), which is calculated from the parameters of the 100-min incubation. Additionally, the gross P mineralization rate ($P_{mineralization}$) and the phosphatase activity after five days of incubation are shown. Numbers in brackets depict standard deviations. N.s. means that the values within one column did not differ significantly between the different treatments of one soil.

Soil type	Depth	Treatment	$r_{1\ min}/R$	n	C_p [μg P g^{-1}]	$E_{1\ min}$ [μg P g^{-1}]	$P_{mineralization\ 5\ days}$ [μg P g^{-1} d^{-1}]	$Phosphatase_{5\ days}$ [μg P g^{-1} d^{-1}]
Leptosol	Topsoil	Control	0.23$^{n.s.}$	0.31$^{n.s.}$	2.83$^{n.s.}$	12.3	12.6 (±3.1)	561.7 (±63.3)
		Alanine	0.23$^{n.s.}$	0.31$^{n.s.}$	2.83$^{n.s.}$	12.3	18.8 (±0.8)	898.3 (±153.3)
		Methionine	0.23$^{n.s.}$	0.31$^{n.s.}$	2.82$^{n.s.}$	12.3	6.4 (±0.7)	468.3 (±56.7)
		Glucose	0.23$^{n.s.}$	0.31$^{n.s.}$	2.83$^{n.s.}$	12.3	19.9 (±1.9)	486.7 (±103.3)
Leptosol	Subsoil	Control	0.06$^{n.s.}$	0.23$^{n.s.}$	2.50$^{n.s.}$	41.6	0.9 (±0.1)	78.3 (±5.0)
		Alanine	0.06$^{n.s.}$	0.23$^{n.s.}$	2.50$^{n.s.}$	41.6	4.8 (±0.6)	421.7 (±8.3)
		Methionine	0.05$^{n.s.}$	0.23$^{n.s.}$	2.50$^{n.s.}$	41.6	1.3 (±0.1)	61.7 (±3.3)
		Glucose	0.06$^{n.s.}$	0.23$^{n.s.}$	2.50$^{n.s.}$	41.6	5.5 (±0.8)	141.7 (±6.7)
Podzol	Topsoil	Control	0.96$^{n.s.}$	0.01$^{n.s.}$	2.41$^{n.s.}$	2.5	0.1 (±0.0)	80.0 (±16.7)
		Alanine	0.95$^{n.s.}$	0.01$^{n.s.}$	2.41$^{n.s.}$	2.5	3.1 (±0.5)	435.0 (±55.0)
		Methionine	0.94$^{n.s.}$	0.01$^{n.s.}$	2.40$^{n.s.}$	2.5	0.1 (±0.0)	60.0(±5.0)
		Glucose	0.96$^{n.s.}$	0.01$^{n.s.}$	2.40$^{n.s.}$	2.5	2.0 (±0.2)	248.3 (±16.7)
Podzol	Subsoil	Control	0.64$^{n.s.}$	0.19$^{n.s.}$	2.94$^{n.s.}$	4.6	0.9 (±0.3)	80.0 (±13.3)
		Alanine	0.62$^{n.s.}$	0.19$^{n.s.}$	2.94$^{n.s.}$	4.6	3.9 (±0.2)	238.3 (±10.0)
		Methionine	0.64$^{n.s.}$	0.19$^{n.s.}$	2.93$^{n.s.}$	4.6	0.9 (±0.1)	95.0 (±45.0)
		Glucose	0.62$^{n.s.}$	0.19$^{n.s.}$	2.94$^{n.s.}$	4.6	1.7 (±0.1)	171.7 (±33.3)
Cambisol	Topsoil	Control	0.21$^{n.s.}$	0.36$^{n.s.}$	2.52$^{n.s.}$	12.0	1.2 (±0.2)	330.0 (±31.7)
		Alanine	0.20$^{n.s.}$	0.36$^{n.s.}$	2.53$^{n.s.}$	12.0	1.7 (±0.1)	411.7 (±18.3)
		Methionine	0.21$^{n.s.}$	0.36$^{n.s.}$	2.52$^{n.s.}$	12.0	1.1 (±0.1)	225.0 (±73.3)
		Glucose	0.22$^{n.s.}$	0.36$^{n.s.}$	2.54$^{n.s.}$	12.0	1.4 (±0.1)	305.0 (±71.7)

three replicates, four treatments. Alanine, methionine and glucose were added in 1 ml distilled H_2O as in the ^{33}P dilution experiment. Soils were incubated at 20 °C for 10 days. Daily, a subsample of 1 g dry mass equivalent was weighted into a jar and made up to 100 g with sterile deionized water. A homogeneous suspension was obtained by sonicating the sample for 3 min. Aliquots of 100 μL were dispensed into 96-well black microplates (Brand), 50 μL of sterile deionized H_2O, and 150 μL of 100 μM substrate solution were added giving a final substrate concentration of 50 μM 4-methylumbelliferyl-phosphate. Plates were incubated for 3 h at 20 °C. Fluorescence was measured after 30, 69, 90, 120, and 160 min on a multilabel counter (Victor^3, Perkin Elmer) with 355 nm excitation and 460 nm emission wavelengths. Enzyme activity was calculated from the linear increase in fluorescence over time. Fluorescence was corrected for quenching of soils and for fluorescence of the substrate according to German et al. (2011). Methylumbelliferon was used for preparation of the calibration standards.

2.8. Statistics

Homogeneity of variance was tested by the Levene-test with a positive result. Differences in ^{33}P recovery and total P in the microbial biomass were tested by ANOVA followed by Duncan-test using the software SPSS18.0, where $\alpha < 0.05$ was considered as the threshold value for significance. Linear regressions were also calculated to derive the parameter n with SPSS18.0 where $\alpha < 0.05$ was considered as the threshold value for significance.

3. Results

3.1. ^{33}P dilution

Addition of glucose, alanine, or methionine did not significantly affect the ^{33}P dilution during 100 min (Table 2). The difference between the modeled and the measured decrease in ^{33}P activity in the soil solution ($r(t)/R$) was largest in the Podzol, indicating a relatively high contribution of biochemical processes to the ^{33}P isotopic dilution in this soil (Fig. 1). P_i concentration in the soil solution did not change significantly over time in the Cambisol (all treatments) and in the topsoil of the Leptosol, but increased in the top- and subsoils of the Podzol (all treatments) as well as in the subsoil of the Leptosol treated with alanine or glucose after seven days of incubation (Appendix 2). The amount of isotopically exchanged P (E) in the untreated soils (control) after 1 min decreased in the order Leptosol subsoil > Leptosol topsoil > Cambisol topsoil > Podzol subsoil > Podzol topsoil (Table 2). In the soils treated with alanine and glucose, E increased more strongly during the incubation than in the control soils (Fig. 2). After five days of incubation, the highest absolute increase in the gross P mineralization rate ($P_{mineralization}$) due to LMWOS addition was observed in the topsoil of the Leptosol (Table 2). The highest relative changes in $P_{mineralization}$ after five days of incubation were observed in the topsoil of the Podzol after alanine and glucose addition (increases by a factor of 31 and 20, Table 2). Additions of methionine had no effect on $P_{mineralization}$ in most soils, except for a small increase in $P_{mineralization}$ in the subsoil of the Leptosol after five days of incubation (Table 2).

3.2. Phosphatase activity

Alanine and glucose increased phosphatase activity in all soils, except for the topsoil of the Leptosol that only showed increases due to alanine addition (Fig. 3). Alanine led to significantly higher phosphatase activities than glucose in all soils. Strongest increases in phosphatase activity – by a factor of 6 – were observed in the subsoil of the Leptosol and the topsoil of the Podzol treated with alanine (Fig. 3B and C). Strongest increases in phosphatase activities due to glucose addition of up to a factor of 4 were found in the topsoil of the Podzol (Fig. 3C). Methionine addition increased phosphatase activity only after seven days in the topsoil of the Cambisol (Fig. 3E). Phosphatase activities were 24–800 times larger than P mineralization rates (Table 2).

3.3. Total P and ^{33}P in the microbial biomass

The recovery of ^{33}P in the microbial biomass at the end of the incubation experiment was in the range of 8–26% of the initially added ^{33}P (Fig. 4A–C). The highest recoveries were observed for the topsoil of the Leptosol (Fig. 4A). Alanine led to a statistically

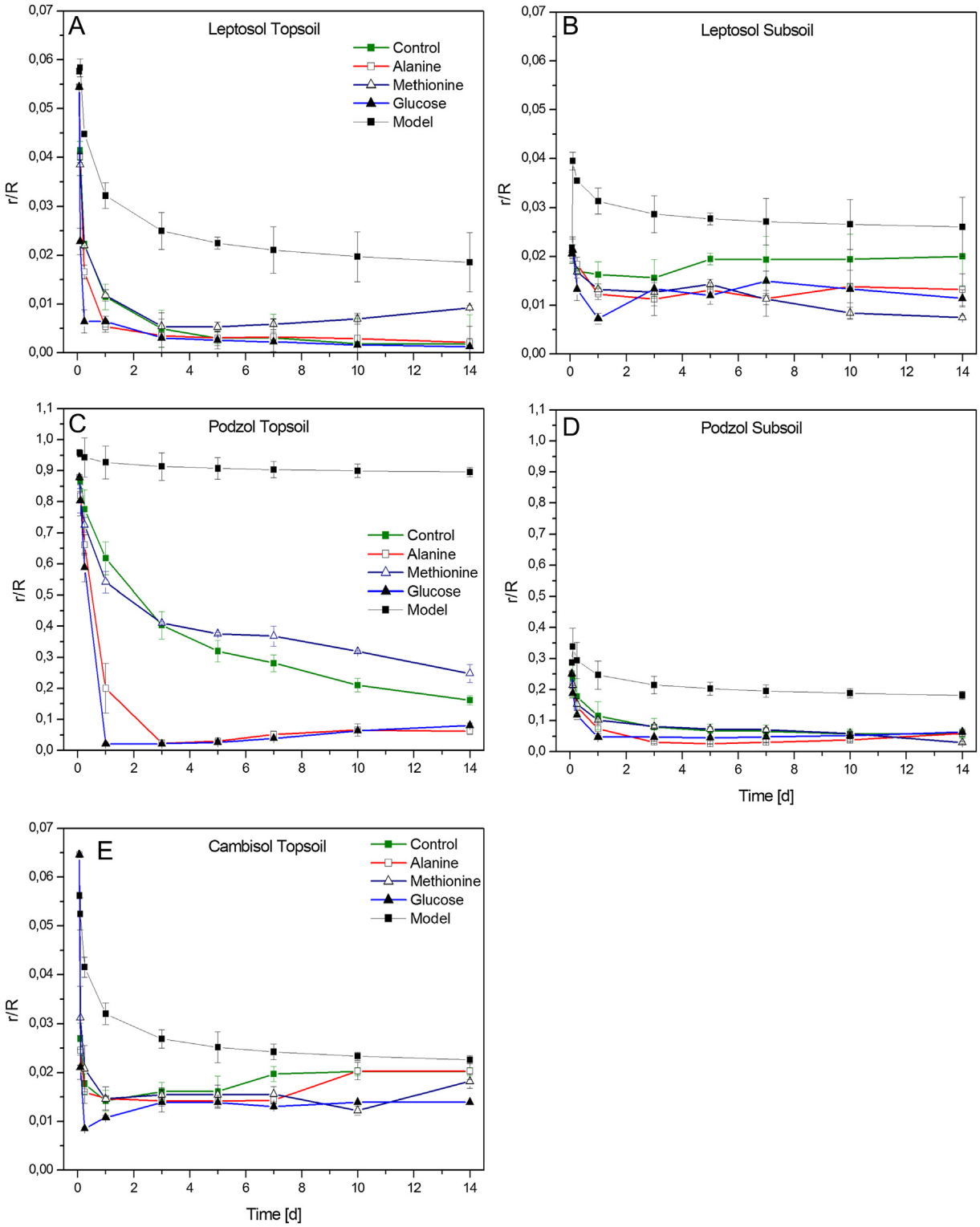

Fig. 1. The proportion ($r(t)/R$) of the initially introduced activity (R) found in soil solution of the top- and subsoil of a Leptosol (A, B), of the top- and subsoil of a Podzol (C, D), and of the topsoil of a Cambisol (E) as a function of time. Beside the measured data for the control and the soils treated with alanine, methionine or glucose, the modeled data is shown that depicts the $r(t)/R$ driven exclusively by physicochemical factors. Error bars depict standard deviations calculated from three independent samples. Notice the difference in scale on the y-axis.

significant, but small increase in ^{33}P recovery in microbial biomass in all soils. Differences in microbial P concentrations between treatments were small and not consistent (Fig. 4D–F). The specific ^{33}P activities did not differ between the treatments in the Cambisol. In the subsoil of the Leptosol treated with alanine and in the subsoil of the Podzol treated with glucose, the specific ^{33}P activities were significantly higher than in the other treatments (Fig. 4G–I).

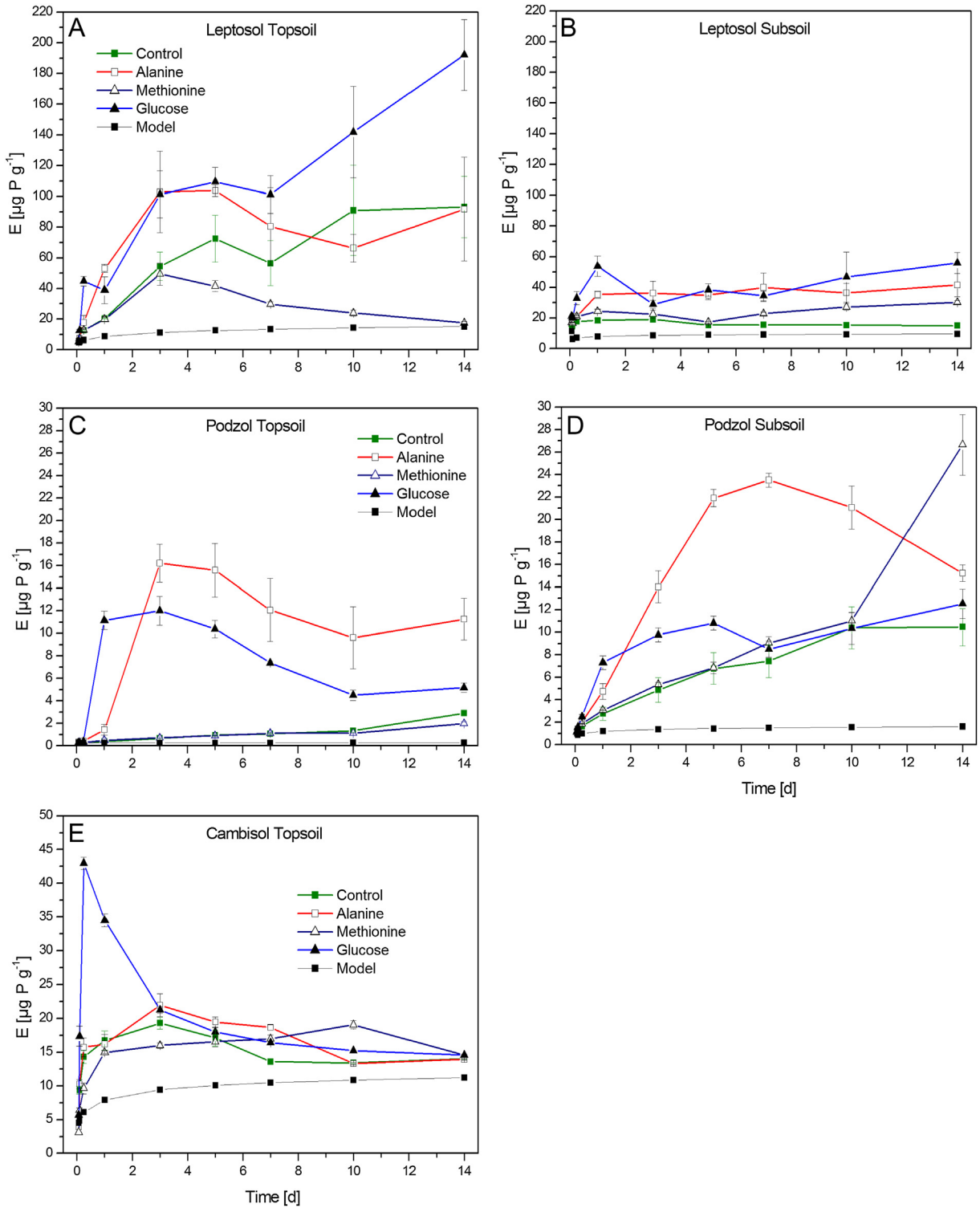

Fig. 2. The amount of the isotopically exchanged P (E) in the top- and subsoil of a Leptosol (A, B), in the top- and subsoil of a Podzol (C, D), and in the topsoil of a Cambisol (E) as a function of time. Beside the measured data for the control and the soils treated with alanine, methionine or glucose, the modeled data is shown. Error bars depict standard deviations calculated from three independent samples. Notice the difference in scale on the *y*-axis.

4. Discussion

4.1. Gross P mineralization

We measured ^{33}P isotopic dilution in soils that were not at steady state because they received rhizosphere-relevant amounts of LMWOS. In previous studies, ^{33}P isotopic dilution was measured in soils that had constant respiration rates, indicating that the soils were at steady state (Oehl et al., 2001; Bünemann et al., 2007; Achat et al., 2009a). However, the 100-min experiment shows that the parameters of the isotopic exchange (Table 2) were not significantly affected by the LMWOS additions during the first

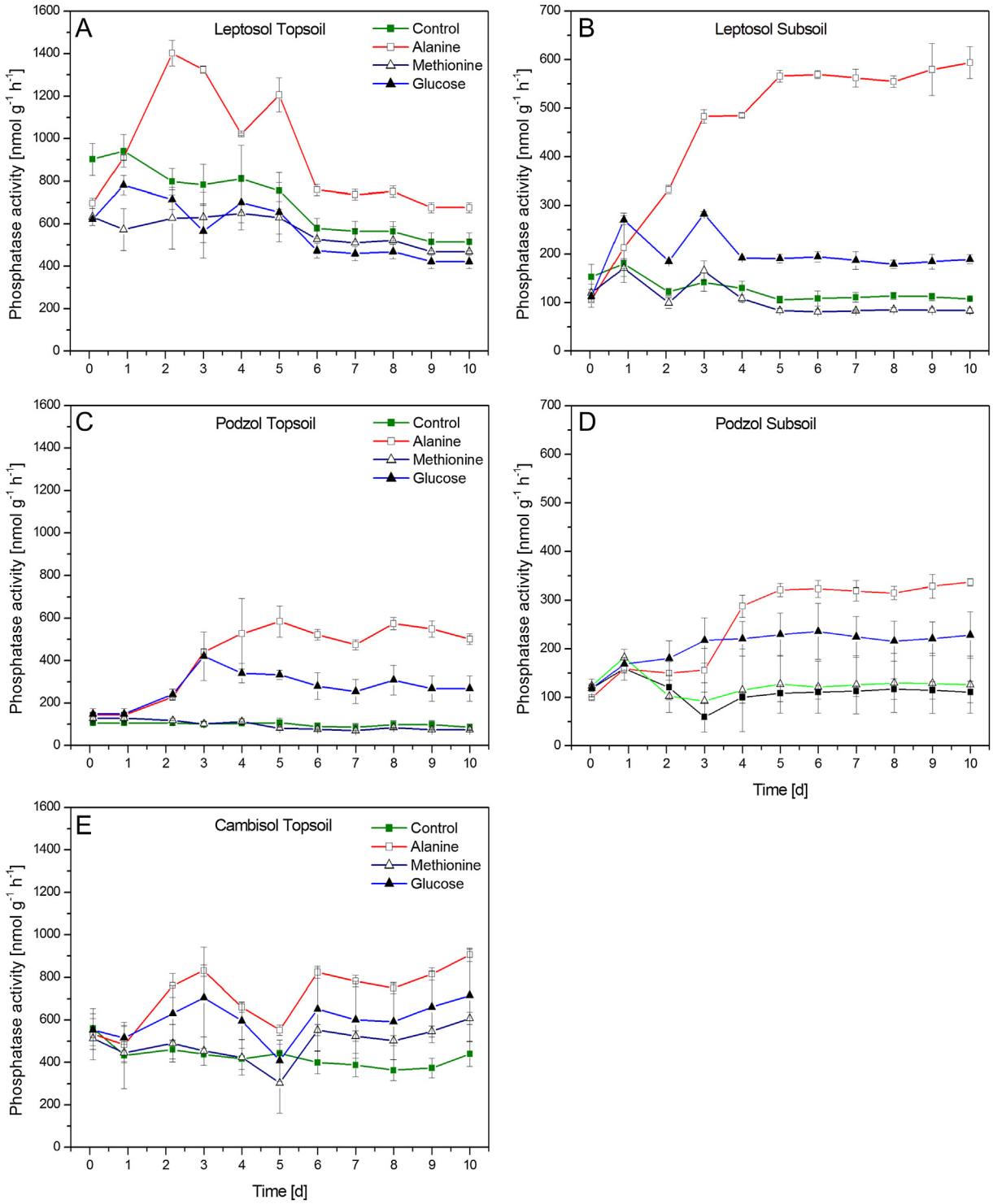

Fig. 3. Phosphatase activity in the top- and subsoil of a Leptosol (A, B), in the top- and subsoil of a Podzol (C, D), and in the topsoil of the Cambisol (E) as a function of time after the addition of alanine, methionine or glucose. Error bars depict standard deviations calculated from three independent samples. Notice the difference in scale on the *y*-axis.

100 min of the incubation, suggesting that the same value of the modeled *E* can be used for the soils treated with LMWOS as for the control soils. The significantly higher gross P mineralization rates ($P_{mineralization}$) due to alanine and glucose addition after five days of incubation (Table 2) indicate that these LMWOS that constitute an important part of root exudates stimulate gross P mineralization. The changing P_i concentrations in the soil solution in the second week of the incubation (Appendix 2) call into question the validity of the modeled isotopic exchange for the second half of the incubation.

A longer lag-phase in response to alanine than to glucose was found for gross P mineralization (Fig. 2). This finding is in agreement with studies that reported longer lag-phases in organic C mineralization after the addition of amino acids than after the addition of monosaccharides (Marstorp, 1996; Hamer and Marschner, 2004). The reason for the shorter lag-phase in

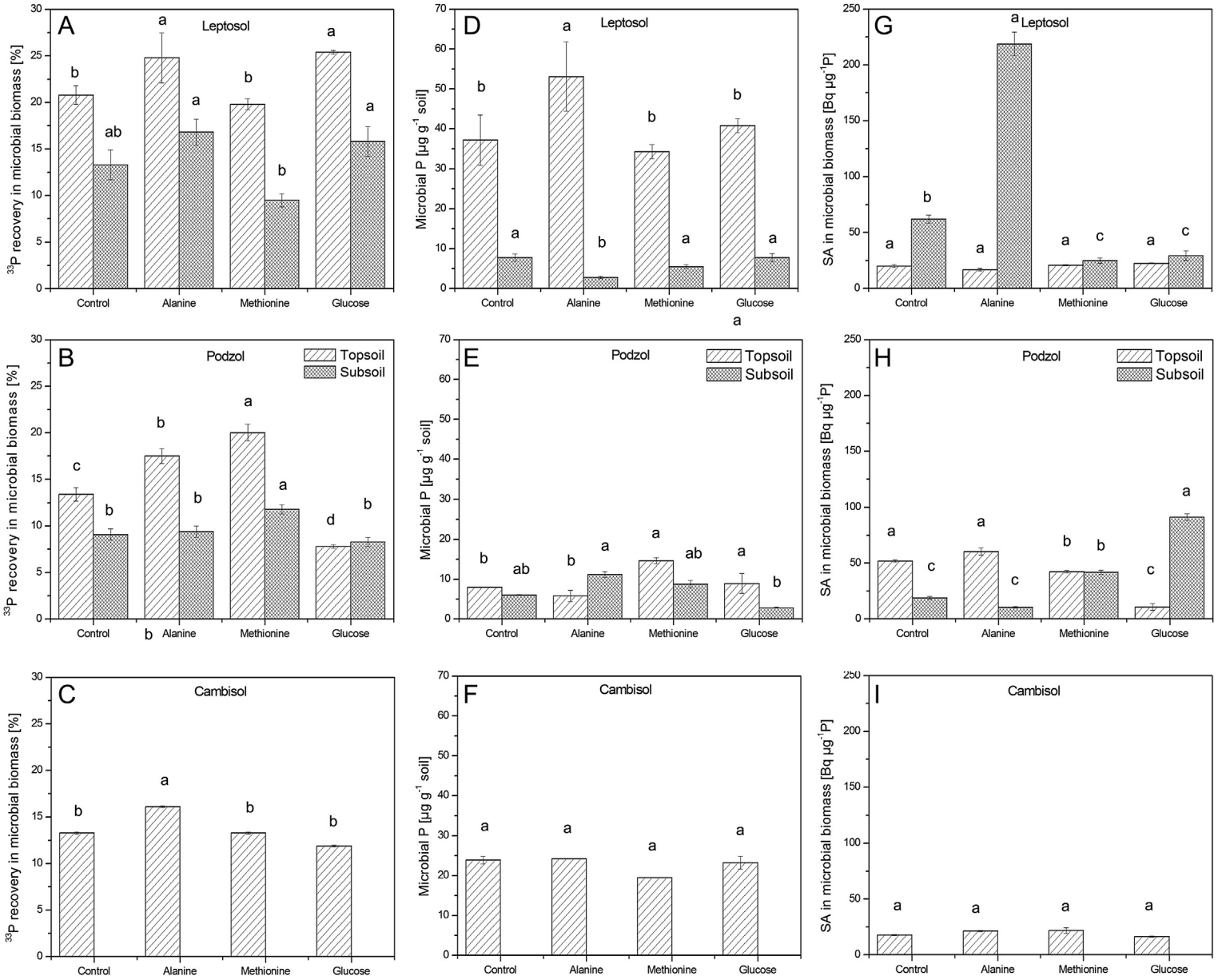

Fig. 4. Recovery of ^{33}P in the microbial biomass (A–C), microbial P (D–F), and specific activity (SA) of the microbial biomass (G–I), in the subsoil and topsoil of Leptosol (A, D, G), Podzol (B, E, H), and in the topsoil of Cambisol (C, F, I) after 15 days of incubation with alanine, methionine or glucose. Letters indicate results of the Duncan-test that was applied separately on each soil horizon. Error bars depict standard deviations calculated from three independent samples.

response to monosaccharides is that microorganisms use a part of the monosaccharides directly as an energy source, while amino acids serve as C and – more importantly – N source (Hamer and Marschner, 2004). The N contained in alanine is most likely also the reason why phosphatase activity was more strongly increased by alanine than by glucose. This finding is in agreement with increased extracellular phosphatase activities caused by atmospheric N depositions (Saiya-Cork et al., 2002). Methionine, in contrast, had little or no effect on gross P mineralization and phosphatase activity (Figs. 2 and 3). The reason for this weak effect of methionine is most likely the C–S bond that impedes mineralization. This observation is supported by results from Fitzgerald and Hale (1988) and Hale and Fitzgerald (1990) who used ^{35}S labeled methionine and cysteine to study the decomposition of S containing amino acids in forest soils. They found that methionine was mostly incorporated into the organic matter instead of being mineralized.

The highest absolute increase in the gross P mineralization rate ($P_{mineralization}$) was observed in the topsoil of the Leptosol (Table 2), which can be attributed to the large microbial biomass in this soil (Table 1). However, the largest relative increase in microbial P mineralization caused by addition of root exudates was observed in the topsoil of the Podzol (Table 2). The reason for this could be that the organic matter in the Podzol is relatively recalcitrant as indicated by the high C/N ratio (Table 1), which might cause C limitation of microorganisms. Consequently, microorganisms might react more strongly to additions of LMWOS than in soils where C is more easily available.

The observation that phosphatase activity was 24–800 times higher than the gross P mineralization rate ($P_{mineralization}$) (Table 2) shows that only a relatively small proportion of the enzyme activity translates into gross P mineralization. In the topsoil of the Podzol the relative difference between phosphatase activity and $P_{mineralization}$ was highest (Table 2), which can be attributed to the low P concentration of this soil.

4.2. Microbial P immobilization

We observed relatively high ^{33}P incorporation into the microbial biomass of 8–26% of the initially added ^{33}P in all soils and treatments after 15 days of incubation (Fig. 4A–C). Similarly, recoveries of 2–25% of ^{33}P after two days of incubation have been found in Oxisols (Oberson et al., 2001). McLaughlin et al. (1988) reported a recovery of 25% of ^{33}P from the microbial pool after seven days. In temperate grasslands, Bünemann et al. (2012) described ^{33}P incorporation between 0 and 37% after 100 min of incubation depending on the P status of the soils; while in Podzols under forest, Achat et al. (2009b) found that 34% of ^{33}P was incorporated in the microbial biomass during one day. The high rates of ^{33}P incorporation in the microbial biomass indicate that microbes can take up large amounts of bioavailable P within a short period of time. LMWOS additions did not consistently affect ^{33}P recovery in the microbial biomass. However, it has to be considered that microbial P was determined at the end of the incubation. Thus, we cannot exclude that microorganisms took up P at a higher rate due to LMWOS addition in the beginning of the incubation and subsequently released it. However, even if on the short term P was immobilized in the microbial biomass it was eventually released as indicated by the microbial P concentrations and microbial ^{33}P activities that were not increased due to LMWOS two weeks after their addition (Fig. 4). Microorganisms in the rhizosphere have recently been described to immobilize N more rapidly than plants, and subsequently release it slowly (Kuzyakov and Xu, 2013). Similarly, a first rapid immobilization of P in the microbial biomass and a subsequent P release could eventually be beneficial for plants.

It is also possible that the microbial P did not increase due to LMWOS during the incubation, and that the microorganisms only mobilized P at higher rates when receiving root exudates without changing the rate of P uptake, which results in a net P mineralization. Taken together, the release of root exudates could be a strategy of plants to render P available from soils by increasing microbial mineralization. The reason why microbes mineralize P, but do not take it up could be that microbial P mineralization is driven by the microorganisms' need for C rather than for P in temperate forest soils as reported by Spohn and Kuzyakov (2013). This interpretation is also in accordance with the finding that microorganisms in Podzols under forest mainly mineralize P through decomposition of soil organic matter (Achat et al., 2010, 2012).

4.3. Conclusions

With respect to our hypothesis we conclude that glucose and alanine stimulated microbial gross P mineralization and phosphatase activity. In contrast, methionine had little effect on both microbial gross P mineralization and phosphatase activity, which can be attributed to the C–S bond in the molecule. A stronger stimulation of phosphatase activity by alanine than by glucose can most likely be ascribed to the N contained in alanine. Our results show that alanine and glucose can stimulate microbial gross P mineralization and phosphatase activity, and that structure and stoichiometry of root exudates significantly shape the extent of this stimulation. In contrast, microbial P immobilization was not consistently changed 15 days after root exudate addition. Taken together, these findings suggest that the release of root exudates could be a strategy of plants to increase P availability in soils by increasing microbial mineralization of organic P.

Acknowledgments

We would like to thank Karin Schmidt and Thomas Klein for technical assistance. We thank the Laboratory for Radioisotopes (LARI), Georg-August-University Göttingen, for providing technical support and working space. The DAAD is appreciated for granting a scholarship to Anton Ermak. We also would like to thank two anonymous reviewers for their helpful comments on an earlier draft of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.05.028.

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