



Subsoil biogeochemical properties induce shifts in carbon allocation pattern and soil C dynamics in wheat

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Abstract

Aims Microbial turnover processes are typically restricted by low substrate availability in the subsoil. We hypothesized that SOM decomposition increases with plant density and decreases with N fertilization. We expected a greater rate of C allocation to the rhizosphere in the topsoil than in the subsoil treatments.

Methods In order to simulate different degrees of rhizodeposition, wheat was planted in pots at four different densities. The plants were continuously labeled with ^{13}C -depleted CO_2 . Soil CO_2 efflux was partitioned for root- and SOM-derived CO_2 . Moreover, we

determined the enzyme kinetics by measuring catalytic efficiency and enzyme stoichiometry in both topsoil and subsoil.

Results Shoot biomass and the shoot to root ratio were significantly higher for plants grown in the topsoil compared with the subsoil, which demonstrated higher relative C allocation to root biomass in the subsoil treatment. Despite the similar size of the rhizosphere, root-derived CO_2 was always higher in the topsoil compared with the subsoil treatment, indicating enhanced root exudation. Effect sizes of all enzyme activities showed stronger magnitudes for the subsoil treatments. This was in line with a two-times increase of the effect size of SOM decomposition in the subsoil relative to topsoil.

Conclusions Overall, the plants in the subsoil treatments allocated more C to root biomass, less C to shoot biomass, and substantially less C to root exudates. However, the effect sizes of both SOM decomposition and enzyme activities were higher in the subsoil than in the topsoil, reflecting a stronger sensitivity to C inputs.

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Introduction

Conventionally managed agricultural soils are characterized by low soil organic carbon (SOC) stocks and high mineral nitrogen (N) contents due to the addition of inorganic fertilizers. Crops take up about 30–50% of N

applied in inorganic form (Tilman et al. 2002). In arable systems with external input of mineral fertilizers, the topsoil is more relevant for nutrient acquisition of crops than the subsoil (Hirte et al. 2018; Kautz et al. 2013) because the majority of root biomass is located in the topsoil, especially in agricultural lands. Therefore, research has been focused on topsoil rather than subsoil processes, and topsoil SOC dynamics are better studied than subsoil SOC dynamics (Salomé et al. 2010). However, subsoil C represents a large reservoir of organic C. SOC in subsoils is primarily stabilized due to selective preservation from microbial decomposition (Rumpel et al. 2004; Wordell-Dietrich et al. 2017) and is controlled by the input and availability of fresh substrates (Fontaine et al. 2007; Guenet et al. 2012; Wordell-Dietrich et al. 2017). Assimilated C is quickly transferred to belowground pools through rhizosphere processes (Cheng and Gershenson 2007; Kuzyakov et al. 2001; Ostle et al. 2000). The C input from deep rooting plants (Lorenz and Lal 2005; Schenk and Jackson 2002) and leached dissolved organic C provide a notable resources for microorganisms in subsoils (Kalbitz et al. 2000). The acquisition of subsoil SOC may support sustainable agricultural practices especially for nutrient and water acquisition when topsoils run dry and diffusion gradients are small (Lorenz and Lal 2005; Lynch and Wojciechowski 2015; Schenk and Jackson 2002).

Plants allocate photoassimilated C to above- and belowground organs to provide C and energy for metabolism, growth, and storage pools (Kuzyakov and Cheng 2001; Larcher 2003). Plant leaves produce photosynthates and plant stems distribute these carbohydrates throughout the plant (Smith and Dukes 2013). In general, the assimilation rate of C limits root growth. By contrast, the growth of leaves is limited by root-supplied water and nutrients. The belowground C pool is regulated by root-associated C fluxes (Cheng and Gershenson 2007; Schimel 1995).

In addition to root litter and dissolved organic C, the main source of C input into subsoils is rhizodeposits of deep-rooting plants. Rhizodeposition is to a large extent driven by photosynthesis (Gavrichkova et al. 2010; Kuzyakov and Gavrichkova 2010), which fosters microbial growth (Loeppmann et al. 2018; Panikov 1995; Blagodatskaya et al. 2009), enzyme production (Loeppmann et al. 2016a), and decomposition of organic compounds (Paterson 2003). Therefore, rhizodeposition may be more important in the subsoil where SOC is limited. Root exudation is known to increase with

planting density, because more C is assimilated and allocated belowground. Photosynthesis also affects root and rhizomicrobial respiration, which may depend more on internal circadian cycles in regulating exudation rather than on sunlight directly (Bahn et al. 2013; Gavrichkova and Kuzyakov 2017). Belowground CO₂ efflux can be partitioned into two distinct processes: (1) rhizosphere respiration or root-derived CO₂, including root respiration and microbial respiration resulting from the mineralization of living root material, and (2) microbial decomposition of soil organic compounds, or soil-derived CO₂. If rhizosphere processes are closely connected to plant photosynthesis and rhizodeposition (Hoberg et al. 2001; Kuzyakov and Cheng 2001), this synchronicity may be beneficial to plants. Here, we address the ways in which plant-soil interactions may affect C allocation to rhizodeposits and root growth in topsoil and subsoil.

The quantity of enzymes produced in the rhizosphere correlates to the activity level of rhizomicrobial populations (Burns 1982). The production of extracellular enzymes by plants, microbes, and soil fauna in both topsoil and subsoil is regulated by nutrient availability (Jones et al. 2018; Liang et al. 2019) and energy demand (Heuck et al. 2015; Sinsabaugh et al. 2009). Therefore, enzymatic activity is an excellent indicator of SOM decomposition and is closely interrelated with soil quality (Bending et al. 2004; Paudel et al. 2011). Tillage and fertilization increase soil aeration and N availability which enhances SOM decomposition, organic N mineralization, and nitrification. However, Zang et al. (2017) recently found that N fertilization increased the assimilation of rhizosphere C into microbial biomass, increased rhizomicrobial turnover rates, and reduced the rate of SOM decomposition. The effects of N fertilization on rhizomicrobial enzymatic catalytic efficiency and stoichiometry under wheat production are largely unknown in both the topsoil and the subsoil.

In a pot experiment, we planted spring wheat in different densities either in topsoil or in subsoil and conducted a continuous ¹³C-CO₂ labeling in order to partition soil CO₂ efflux. With increasing planting density, we expected higher root and shoot biomass and greater rhizodeposition. We anticipated that this would enhance microbial biomass and SOM decomposition for both the topsoil and subsoil treatments. A second set of treatments with ten plants per pot received nitrogen fertilizer since SOM decomposition and microbial activity strongly depend on the availability of mineral N in the soil. We hypothesized that SOM decomposition

increases with plant density and decreases with N fertilization. We expected a higher amount of aboveground biomass, a greater rate of photosynthesis, and a greater rate of C allocation to the rhizosphere (respiration and exudation) in the topsoil treatments.

We integrated the activities of enzymes involved in the same process as indicators of organic matter degradation and nutrient transformation. It is assumed that the sum of major C-acquiring enzyme activities is a better indicator of total C acquisition than e.g. BG solely (Bell et al. 2014; Loeppmann et al. 2016b; Nannipieri et al. 2012). Thus, enzyme activity ratios were used as a tool for examining relative allocation to energy versus nutrient acquisition (Hill et al. 2014; Moorhead et al. 2013). Extracellular hydrolytic enzyme stoichiometry and the catalytic efficiency were used to explain differences in the exploitation of resources between topsoil and subsoil (Gianfreda et al. 1995; Koshland 2002; Nannipieri et al. 2012). Microbial biomass C was determined, since it is the crucial pool facilitating the decomposition of organic material. This study addresses some of the underlying mechanisms driving plant-soil interactions, especially nutrient acquisition and competition in topsoil and subsoil systems.

Material and methods

Plants and soil

To manipulate root C input, we planted spring wheat (*Triticum aestivum* L.) with the following planting densities (Table 1): very low (2 plants pot^{-1} ; T₂), low (5 plants pot^{-1} ; T₅), common for agricultural systems (10 plants pot^{-1} ; T₁₀), and high (20 plants pot^{-1} ; T₂₀). In addition, unplanted controls for each the topsoil and subsoil treatments were prepared. A second set of the T₁₀ treatment received N fertilizer (Table 1). Plants were grown in PVC pots (15 cm diameter, 40 cm height, equipped with an inlet tube at the bottom for aeration and soil CO₂ trapping). A nylon bag filled with 1500 g sand was placed at the bottom of each pot to improve air circulation. Soil from two contrasting horizons was used in this experiment: (1) topsoil (0–20 cm, Ap horizon), (2) subsoil (70–90 cm, B horizon). The soil was taken from an organic farm at the University of California, Santa Cruz. The soil is classified as a sandy loam Mollisol. The Ap horizon had an average C to N ratio of 9.8, a $\delta^{13}\text{C}$ value of -23.3‰ , and a pH value of 5.8.

The B horizon showed an average C to N ratio of 7.8, a $\delta^{13}\text{C}$ value of -17.2‰ , and a pH value of 6.1. In order to achieve a high degree of soil homogeneity and to reduce variability among treatments and replicates, the soil was air-dried then sieved (<4 mm). All pots were filled with 6 kg dry weight soil equivalent and wetted with deionized water to 80% of the water holding capacity, estimated separately for filled topsoil and subsoil pots.

To minimize the influence of a high CO₂ efflux due to soil disturbance by sieving, we pre-incubated the soils after water addition in the greenhouse for 2 weeks before the start of the experiment. The CO₂ concentration during pre-incubation was monitored by an infrared gas analyzer (Li-820 CO₂ gas analyzer Biosciences serial O/I). After 2 weeks of pre-incubation, the water lost by evapotranspiration was refilled based on the weight of the filled pots. Ammonium nitrate was dissolved in water and added at a rate of 15 g N m^{-2} (0.3 g N pot^{-1}) with the first water amendment after pre-incubation to half of the unplanted pots and to additional pots in the T₁₀ treatment. For planting, the wheat seeds were presoaked overnight. Twice as many seeds were sown for each treatment, and the seedlings were thinned to the respective number after emergence. The soil moisture content was measured gravimetrically and adjusted daily to 80% of the water holding capacity. The location of the pots in the greenhouse was changed twice a week by mixing them randomly to guarantee similar growing conditions for the plants. Artificial lighting (1100-W lights, P.L. Light Systems, Beamsville, ON) was used to ensure an adequate energy flux during the experiment. The light intensity was kept above 900 W m^{-2} and the photoperiod was set from 4:30 a.m. to 4:30 p.m. The relative air humidity was kept constant at 45% by a dehumidifier (Kenmore Elite 70 pint, Sears, Chicago, IL, USA). The inlet tube at the bottom of each pot was connected to an aquarium pump to aerate the pots. This was done two times during the dark period to avoid contamination of the growth chamber $\delta^{13}\text{C}$ signal with that of soil-derived CO₂ during the assimilation period.

¹³CO₂ continuous labeling

The experiment was conducted in a greenhouse equipped with supplies for continuous labeling with ¹³C-depleted CO₂ (Cheng and Dijkstra 2007; Zhu and Cheng 2012; Pausch et al. 2013, 2016). Plants were

Table 1 Plant components, rhizosphere soil, and microbial biomass C are given for T = topsoil and S = subsoil. The subscript number gives the number of plants per pot; +N indicates treatments with mineral N fertilizer addition. Lowercase letters denote significant differences ($P < 0.05$) between plant densities and the asterisk indicates differences between top- and subsoil

Soil type	Code	Treatments		Plant biomass				$\delta^{13}\text{C}$		C to N ratio				MBC (mg kg ⁻¹ soil)
		Plant density (plants pot ⁻¹)	N fertilizer	Replicates	Shoot (g DW pot ⁻¹)	Root (g DW pot ⁻¹)	Shoot/root (-)	Shoot (%)	Root (%)	Shoot (-)	Root (-)	Rhizosphere soil (-)		
Topsoil	T ₀	0		5										299 ± 34*
	T _{0+N}	0	+N	5										279 ± 36*
	T ₂	2		4	0.8 ± 0.1c*	0.1 ± 0.0c	7.5 ± 2.2*	-40.1 ± 0.1*	-38.0 ± 0.2ab*	6.2 ± 0.3b	13.2 ± 1.3d	10.0 ± 0.1*		325 ± 12*
	T ₅	5		4	1.3 ± 0.2c*	0.2 ± 0.0bc*	6.5 ± 1.0*	-40.0 ± 0.1*	-37.9 ± 0.2ab	6.3 ± 0.3b	15.1 ± 2.0cd	10.2 ± 0.2*		313 ± 21*
	T ₁₀	10		4	2.9 ± 0.3b*	0.6 ± 0.1b*	5.4 ± 0.6*	-40.2 ± 0.1*	-38.1 ± 0.2ab*	7.9 ± 1.1b	17.6 ± 1.6cb	9.6 ± 0.3*		339 ± 23*
	T _{10+N}	10	+N	4	3.4 ± 0.3ab*	0.5 ± 0.1bc*	7.4 ± 0.5*	-40.0 ± 0.1*	-37.5 ± 0.3a	6.0 ± 0.3b	14.1 ± 1.1d	9.9 ± 0.2*		280 ± 9*
	T ₂₀	20		4	4.3 ± 0.2a*	1.1 ± 0.1a	4.0 ± 0.3*	-40.1 ± 0.1*	-38.4 ± 0.2b*	10.7 ± 2.2a	21.8 ± 1.4a	10.4 ± 0.3*		320 ± 20*
	Subsoil	S ₀	0		5									173 ± 8
	S _{0+N}	0	+N	5										189 ± 6
	S ₂	2		5	0.2 ± 0.0d	0.2 ± 0.0c	0.9 ± 0.1	-39.2 ± 0.1b	-36.8 ± 0.3	8.3 ± 0.2	18.9 ± 0.5	7.5 ± 0.1		159 ± 15
S ₅	5		5	0.5 ± 0.0cd	0.4 ± 0.0c	1.2 ± 0.1	-39.0 ± 0.1ab	-37.5 ± 0.2	9.2 ± 0.6	18.4 ± 0.5	7.3 ± 0.2		180 ± 8	
S ₁₀	10		5	1.0 ± 0.1b	0.8 ± 0.1b	1.2 ± 0.1	-38.9 ± 0.1ab	-37.0 ± 0.1	9.7 ± 0.9	20.8 ± 2.8	7.7 ± 0.1		199 ± 9	
S _{10+N}	10	+N	5	0.8 ± 0.1bc	0.8 ± 0.1b	1.0 ± 0.2	-38.9 ± 0.1ab	-37.1 ± 0.1	8.5 ± 0.2	19.4 ± 3.3	7.2 ± 0.2		184 ± 5	
S ₂₀	20		5	1.6 ± 0.1a	1.3 ± 0.1a	1.2 ± 0.1	-38.8 ± 0.1a	-37.0 ± 0.2	9.8 ± 1.2	18.6 ± 0.6	7.3 ± 0.1		201 ± 9	

continuously, labeled from the emergence of the first leaf until harvest, exposed to ^{13}C -depleted CO_2 ($-18.0 \pm 0.6\%$). Soil CO_2 efflux was measured 5 weeks after planting by means of a closed-circulation CO_2 trapping system (Cheng et al. 2003; Pausch et al. 2013). Soil CO_2 was trapped in 400 mL of 1 M NaOH for 24 h. Afterwards, an aliquot of each NaOH solution was analyzed for total inorganic C using a Shimadzu TOC-5050A Total Organic Carbon Analyzer. Another aliquot was precipitated as SrCO_3 (Harris et al. 1997) and analyzed for $\delta^{13}\text{C}$ by means of a Carlo Elba 1108 elemental analyzer interfaced to a continuous flow isotopic ratio mass spectrometer Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer at the Isotope Facility of University of California, Santa Cruz.

After CO_2 trapping, the pots were destructively harvested. The shoots were cut at the base. The soil of each pot was pulled out and the roots were separated by hand-picking. The soil adhering to the roots was collected by slightly shaking the roots and was defined as rhizosphere. Roots were rinsed under deionized water. Shoots and roots were dried at 60°C for 3 days, weighed, ground in a ball mill, and measured for $\delta^{13}\text{C}$.

Calculation of root- and SOM-derived C

The contribution of CO_2 derived from SOM decomposition ($C_{\text{SOM-derived}}$, $\text{mg C day}^{-1} \text{kg}^{-1}$ soil) to total soil respiration was calculated using linear two-source isotopic mixing model:

$$C_{\text{SOM-DEIVED}} = C_{\text{TOTAL}} \cdot \frac{\delta^{13}\text{C}_{\text{TOTAL}} - \delta^{13}\text{C}_{\text{ROOT-DEIVED}}}{\delta^{13}\text{C}_{\text{SOM-DEIVED}} - \delta^{13}\text{C}_{\text{ROOT-DEIVED}}} \quad (1)$$

$$C_{\text{ROOT-DEIVED}} = C_{\text{TOTAL}} - C_{\text{SOM-DEIVED}} \quad (2)$$

where C_{TOTAL} is the total CO_2 efflux of the planted treatment ($\text{mg C day}^{-1} \text{kg}^{-1}$ soil) and $\delta^{13}\text{C}_{\text{TOTAL}}$ the corresponding $\delta^{13}\text{C}$ value of CO_2 from SOM decomposition measured in the unplanted pots (%). $C_{\text{ROOT-DEIVED}}$ is the root-derived CO_2 in the planted pot ($\text{mg C day}^{-1} \text{kg}^{-1}$ soil) with $\delta^{13}\text{C}_{\text{ROOT-DEIVED}}$ as the corresponding $\delta^{13}\text{C}$ value (%). The isotopic composition of root-derived CO_2 was calculated by the $\delta^{13}\text{C}$ value of the roots plus a fractionation factor of 0.87 (Zhu and Cheng 2011). This fractionation factor was determined

for wheat with a similar experimental setup as compared to the present study.

Determination of soil microbial biomass and mineral nitrogen

Soil microbial biomass C (MBC) was determined on all remaining soil samples by the chloroform fumigation extraction method described by Vance et al. (1987) with the modification that fumigated and non-fumigated soil samples (30 g fresh soil) were extracted for 2 h with 60 mL of 0.5 M K_2SO_4 solution. The samples were filtered and the extracts were analyzed for total organic carbon by means of a Shimadzu TOC-5050A Total Organic Carbon Analyzer. The difference between the extracts of fumigated and non-fumigated samples corrected for a kEC factor of 0.45 (Wu et al. 1990) gives the total amount of microbial biomass C. To determine the water content, 30 g fresh soil was dried at 105°C for 3 days and weighed thereafter.

Soil mineral N (N_{min} ; $\text{NO}_3^- + \text{NH}_4^+$) was extracted from 30 g fresh soil with 60 ml of 2 M KCl solution. Samples were shaken for 2 h and filtered and the extracts were analyzed for NO_3^- and NH_4^+ by a flow injection analyzer (Lachat QuikChem 8000, Milwaukee, WI).

Enzyme kinetics

We determined the enzyme activities of β -cellobiohydrolase (exo-1,4- β -glucanase, EC 3.2.1.91), β -glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), β -xylosidase (EC 3.2.2.27), leucine-aminopeptidase (EC 3.4.11.1), and N-acetyl- β -glucosaminidase (EC.3.2.1.14) respectively. β -Glucosidase (BG), β -cellobiohydrolase (CE), and β -xylosidase (BX) represented enzymes in the C cycle, whereas leucine-aminopeptidases (LE) represented N cycling enzymes. Acid phosphatase (AP) is responsible for substrate utilization in the P cycle. N-Acetyl- β -D-glucosaminidase (Chitinase, NAG) catalyzes the hydrolytic cleavage of the β -1 \rightarrow 4-glycoside bond present in biopolymers of N-acetylglucosamine, primarily in chitin (C and N cycles). Indeed, chitinases are widely distributed in living organisms and are found in fungi, bacteria, parasites, plants, and animals.

We added half a gram of soil to 50 ml sterile water in autoclaved jars and dispersed by an ultrasonic disaggregation (50 J s^{-1} for 120 s; De Cesare et al. 2000).

Aliquots of 50 ml were withdrawn while stirring the suspension and dispensed into 96-well microplates (Brand pureGrade, black). Fifty microliter of buffer was added (0.1 M MES buffer, pH 6.1 for carbohydrases and phosphatase, 0.05 M TRIZMA buffer, pH 7.8 for leucine-aminopeptidase) (Loeppmann et al. 2016b; Marx et al. 2005). Finally, 100 ml of substrate solution was added at a series of concentrations (20, 40, 60, 80, 100, 200, 400 μmol substrate g^{-1} soil). Plates were kept at 22 °C, agitated and measured fluorometrically (excitation 360 nm; emission 450 nm) after 1-h, 2-h, and 3-h incubation using an automated fluorometric plate reader (Wallac 1420, PerkinElmer, Turku, Finland). Fluorescence was converted into an amount of 4-methylumbelliferone or 7-amino-4-methylcoumarin by reference to the fluorescence of standard solutions, which had been prepared in sub-samples of the various soil suspensions. Each replicate was measured as an analytical triplicate. We determined the catalytic efficiency for all measured enzymes (Gianfreda et al. 1995; Koshland 2002; Moscatelli et al. 2012). It reflects the total enzyme catalytic process combining enzyme-substrate complex dissociation and the rate of enzyme-substrate complex formation (Cornish-Bowden 1995; Koshland 2002).

Statistics

The values presented in the figures and tables are given as means \pm standard errors of the means (\pm SEM). A Shapiro-Wilk test was applied to test for Gaussian distribution. We used the Pearson correlation coefficients to interpret the degree of linear relationships. Significant differences of the measured data between top- and subsoil and between the plant density treatments were obtained by analysis of variance (ANOVA) in combination with a post hoc unequal N HSD test. Parameter optimization of enzyme properties was restricted to the applied model equation (Michaelis-Menten) as indicated by maximum values of statistic criteria: r^2 , the fraction of total variation explained by the model defined as ratio of model weighted sum of squares to total weighted sum of squares. Identification of outliers was performed by the ROUT method, based on the false discovery rate (FDR) (Motulsky and Brown 2006). The standardized data (z -scoring) was analyzed by principal component analysis (PCA), using the software PAST 3.14 (Hammer et al. 2001).

Results

Shoot and root biomass, soil respiration, and microbial biomass

The shoot biomass and the shoot to root ratio were higher for plants grown in the topsoil compared with the subsoil (Table 1), which demonstrated higher relative C allocation to roots in subsoil possibly for nutrient acquisition. The shoot to root ratios for the topsoil treatment ranged from 4 to 7.5, whereas those for the subsoil treatments ranged from 0.9 to 1.2. The shoot biomass ranged from 0.8 to 4.3 g DW pot^{-1} in topsoil and from 0.2 to 1.6 g DW pot^{-1} in the subsoil.

Nutrient limitation in the subsoil and better plant growth in topsoil were also indicated by a 3-times steeper slope of the positive linear relationship between root biomass and shoot biomass in the topsoil treatments compared with the subsoil treatments (Fig. 1).

The ratio of rhizosphere soil to total soil increased with plant density but did not differ significantly between top- and subsoil (Fig. 2). Root-derived CO_2 was always higher in the topsoil treatments (Fig. 3A) and had a significant positive correlation with shoot biomass (Fig. 4a). This is also supported by a steeper slope of the linear regression between root biomass and root-derived CO_2 in the topsoil treatments compared with the subsoil treatments (Fig. 4b). The topsoil treatments produced greater amounts of photosynthetically active biomass, allocated more assimilates belowground, and had higher microbial biomass. Overall, the topsoil treatments produced much more CO_2 than the subsoil treatments (Table 1). The most striking result was that wheat plants used much more C for rhizodeposition and respiration (indicated by higher root-derived CO_2) when grown in topsoil compared with subsoil, despite a lower root biomass of plants grown in topsoil.

All planted treatments showed consistently positive priming effects; however, these effects were not statistically significant from zero (Fig. 3B). Although the total SOM-derived CO_2 efflux was significantly higher in topsoil than in subsoil (Fig. 3B), the relative difference in SOM-derived CO_2 between the planted treatment and the respective unplanted control was higher in sub- than in topsoil, reflecting intensified decomposition of organic compounds. However, two-times greater effect size of SOM-

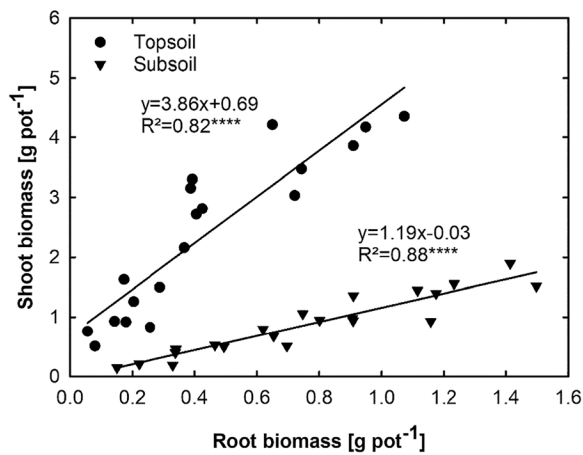


Fig. 1 Strong correlation between shoot and root biomass for both topsoil and subsoil (**** $P \leq 0.0001$)

derived CO_2 was observed in the subsoil than in the topsoil (Fig. 6).

Overall, microbial biomass was higher in the topsoil compared with the subsoil across all planting densities (Table 1). PCA for planted topsoil revealed strong magnitudes of SOM-derived CO_2 , root-derived CO_2 , total CO_2 efflux, and shoot biomass, which are explained by PC1, whereas MBC was best explained by PC2 (not shown).

Enzyme activity and catalytic efficiency

The effect of increasing C input via exudation on extracellular enzyme activities (V_{\max}) was evident in both soils. Especially, in the T_{20} treatment, V_{\max} significantly increased compared with densities < 20 plants per pot (Supplementary Fig. 3). All enzyme activities were higher in the topsoil compared with the subsoil, except for AP (Supplementary Fig. 3). However, the effect sizes of enzyme activities were higher in all plant densities (Fig. 6). In the subsoil, the AP activity significantly increased for both 10 and 20 plants per pot compared with all other treatments (Supplementary Fig. 3). In the topsoil, the AP activity was exclusively high for T_{20} ($P < 0.05$). Most enzyme activities were positively correlated to root biomass (Fig. 5). Regression analyses revealed steeper enzyme activity slopes in planted topsoil than in subsoil, except for AP (Fig. 5). The effect sizes of all enzyme activities were higher in the subsoil than in the topsoil, indicating enhanced sensitivity to C input (Fig. 6).

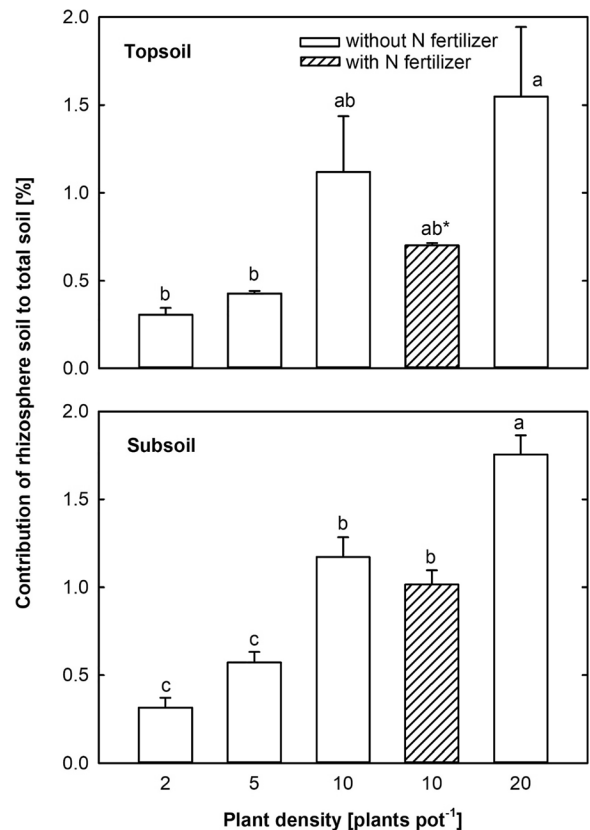


Fig. 2 Contribution of rhizosphere to total soil in topsoil (top) and subsoil (bottom) with increasing plant density. The common plant density in agroecosystems was established with and without addition of mineral N fertilizer (10 plants pot^{-1}). Significant treatment effects were assessed by ANOVA ($P < 0.05$) and indicated by different lowercase letters. The asterisk denotes significant effects between topsoil and subsoil

The proportional enzyme activities of C and P acquiring enzymes varied significantly between the top- and subsoil. Across all plant densities, the C to P ratio was higher in the topsoil (up to 4-fold in T_{10}) compared with subsoils, reflecting stronger P limitation in the subsoil (Supplementary Fig. 4). The proportional enzyme activities of C versus N acquiring enzyme did not show any effect between topsoil and subsoil (Supplementary Fig. 4).

Planting density had the most pronounced effect on the catalytic efficiency (K_a). K_a was strongly depleted exclusively for T_{20} in the topsoil whereas in the subsoil the opposite was the case (Supplementary Fig. 1). Overall, K_a of AP, CE, and BG was lower in subsoil, whereas the K_a of BX, LE, and partly NAG showed almost equal efficiencies in top- and subsoil (Supplementary Fig. 1).

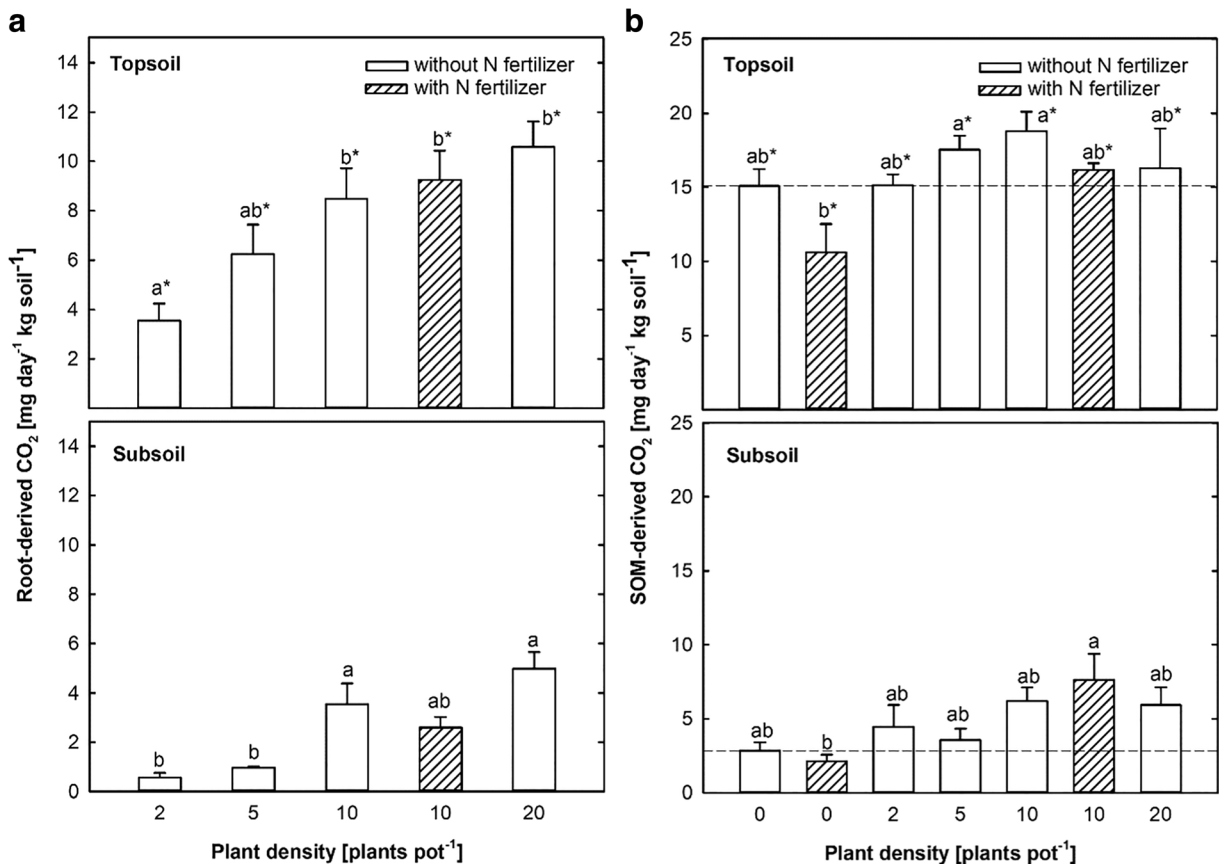


Fig. 3 Root-derived CO₂ (A) and SOM-derived CO₂ (B) for the topsoil and the subsoil treatments; The horizontal dashed line indicates the rhizosphere priming effect. The common plant density in agroecosystem was established with and without addition of

mineral N fertilizer (10 plants pot⁻¹). Significant treatment effects were assessed by ANOVA ($P < 0.05$) and indicated by different lowercase letters. The asterisk denotes significant effects between topsoil and subsoil

Effect of N addition

Mineral N addition significantly reduced the root C to N in all treatments (Table 1). N_{\min} was always higher in the subsoil treatments and increased up to 2-fold with N addition in S_{10} (data not shown), reflecting stronger plant uptake in topsoil at all plant densities. This is in line with a strong non-linear negative relationship between N_{\min} and root biomass, whereas a linear decrease was observed for subsoil (Fig. 7). The catalytic efficiency of acid phosphatase was 5-fold lower in the subsoil compared with the topsoil in the T_{10} N_{\min} treatments (Supplementary Fig. 1).

In summary, wheat altered the allocation of carbon differently in the top- and subsoil treatments in response to the addition of N_{\min} . Wheat allocated more C to root biomass, less to shoot biomass, and, more importantly, substantially less to root-derived CO₂ (respiration of

roots and decomposition of exudates) when grown in the subsoil, resulting in lower microbial activity and SOM decomposition (Fig. 8).

Discussion

Topsoil versus subsoil

Higher enzyme activities corresponded to higher microbial biomass in the topsoil, inducing a strong reduction of mineral N (Table 1, Supplementary Fig. 3, Fig. 7). This enhanced plant shoot production and resulted in a higher shoot to root ratio as compared with plants grown in the subsoil (Table 1, Fig. 1). Moreover, the tendency for greater root biomass production in the subsoil (yet not significant for all of the plant densities) reflected the increased plant need for investments into the roots to

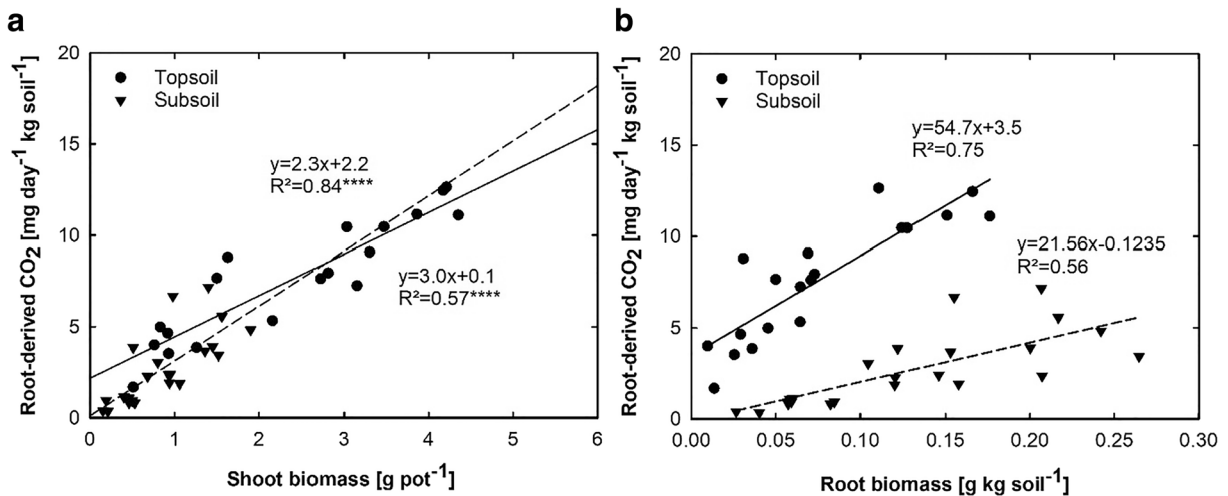


Fig. 4 (a) Almost identical positive linear relationship between root-derived CO₂ and shoot biomass for both the topsoil and the subsoil. (b) Stronger positive linear relationships between root-

derived CO₂ and root biomass for the topsoil compared with the subsoil (**** $P \leq 0.0001$)

increase nutrient acquisition from subsoil (Table 1). Lynch (2007) reported lower shoot growth than root growth when nutrients are scarce. This might be attributed to the functional equilibrium between C allocation and resource capture rates of shoots and roots (Gregory 2006; Pinton et al. 2007). Both shoots and roots obtain resources, which are commonly thought of as a relatively stable ratio. Therefore, root growth is contingent not only on C from the shoot but also on its own acquisition of N (Farrar and Jones 2000; Thornley 1977).

About 50% of photosynthates are translocated belowground in wheat plants at early tillering. Of this, approximately one-half is respired and one-quarter each is recovered in roots and soil (Keith et al. 1986). Above- and belowground processes were found to be closely coupled irrespective of soil horizon. This is demonstrated by the relationship between shoot biomass and root-derived CO₂ for both the top- and subsoil treatments (Fig. 3). These results are consistent with previous findings which suggest that respiration is largely driven by C assimilation via photosynthesis and that photosynthesis modulates the belowground C allocation of plants (El-Sharkawy et al. 1990; Kuzyakov and Gavrichkova 2010). Despite a similar ratio of rhizosphere soil to bulk soil in each treatment (Fig. 2), root-derived CO₂ was consistently and significantly higher in the topsoil compared with the subsoil (Fig. 3). This may indicate that the amount of photosynthetically active biomass is a more influential factor than the amount of root biomass in predicting belowground C allocation. Assuming an

equal amount of root biomass in top- and subsoil, the greater amount of root-derived CO₂ produced in the topsoil, resulted from higher allocation of assimilates to the roots due to greater shoot biomass and therefore higher photosynthesis. A shortage of resources in the rhizosphere is known to cause a shift of C assimilates in favor of the root system relative the shoot (Brouwer 1983). Farrer and Jones (2000) proposed several C allocation mechanisms such as push, pull, and shared control hypothesis. They concluded that there is qualitative and quantitative evidence supporting the hypothesis that control of C flux into roots is shared between the many processes which contribute to whole plant C flux, including those determining the loss of C from the root to soil and its organisms (Farrer and Jones 2000). Top-down, the C allocation to the roots is strongly dependent on the photomorphogenesis and the storage of assimilates, whereas the bottom-up pathway is strongly controlled by the soil nutrient availability and abiotic factors (Farrer and Jones 2000). The shared control hypothesis (Farrer and Jones 2000) may describe the C allocation best in our study.

We obtained a positive linear relationship between root-derived CO₂ and root biomass (Fig. 4b). Field experiments demonstrated that root growth is strongly correlated to rhizodeposition and root-derived CO₂, which supports our findings (Prikryl and Vancura 1980; Remus and Augustin 2016). Crops and grasses have been found to allocate a greater amount of C to roots than to net rhizodeposits. In crop species, net

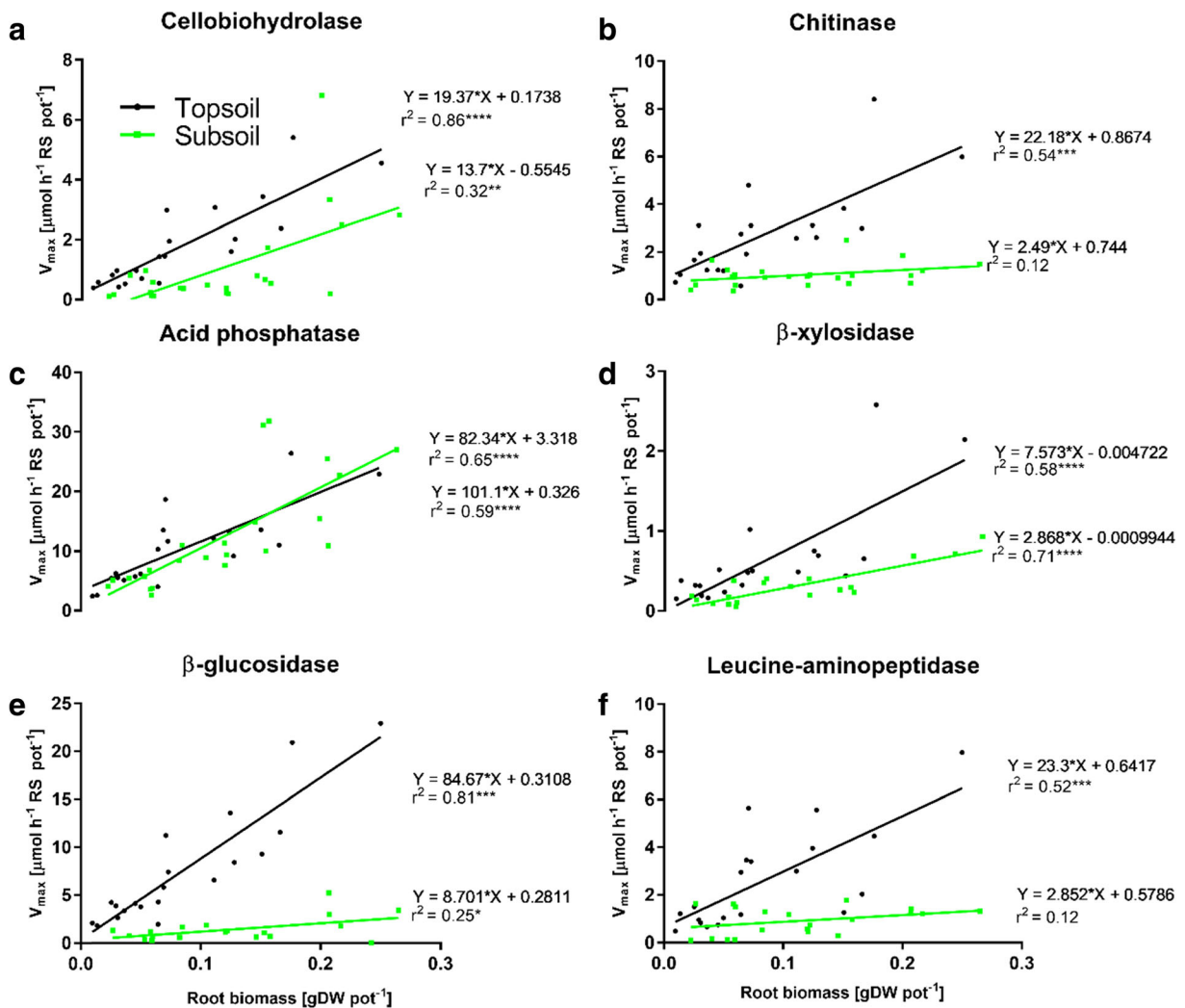


Fig. 5 Linear regression between enzyme activity (V_{\max}) of (a) cellobiohydrolase, (b) chitinase, (c) acid phosphatase, (d) β -xylosidase, (e) β -glucosidase, and (f) leucine-aminopeptidase in

rhizosphere soil (RS) and the root biomass. The asterisk denotes the significant effects (**** $P \leq 0.0001$; *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$)

rhizodeposition accounts for 3% of assimilated C and root-derived CO_2 accounts for 8% of assimilated C (Pausch and Kuzyakov 2017). Net rhizodeposition may remain steady in a system despite increases in gross rhizodeposition if microbial decomposition of rhizodeposition and SOM increases simultaneously via rhizosphere priming (Huo et al. 2017; Pausch et al. 2016; Shahbaz et al. 2018). For this reason, gross rhizodeposition may provide a better measure of changes in C allocation than net rhizodeposition (Pausch et al. 2016). Taking these factors into account, there was no significant change in the rate of SOM decomposition in any of the planted treatments compared with the

unplanted control treatments. Nevertheless, we found a trend of increasing decomposition of organic compounds as plant density increased which suggests that the direction and magnitude of the rhizosphere priming effect may be impacted by the availability of labile root-derived C in the C limited subsoil. Here, higher microbial biomass and higher enzyme production induced intensive decomposition of organic compounds, which resulted in higher SOM-derived CO_2 in the topsoil relative to the subsoil (Fig. 4a) reflecting lower rhizodeposition in subsoil treatments. The turnover rate of recent C pools is higher compared with older ones in deeper soil horizon, and decreases with time after the C

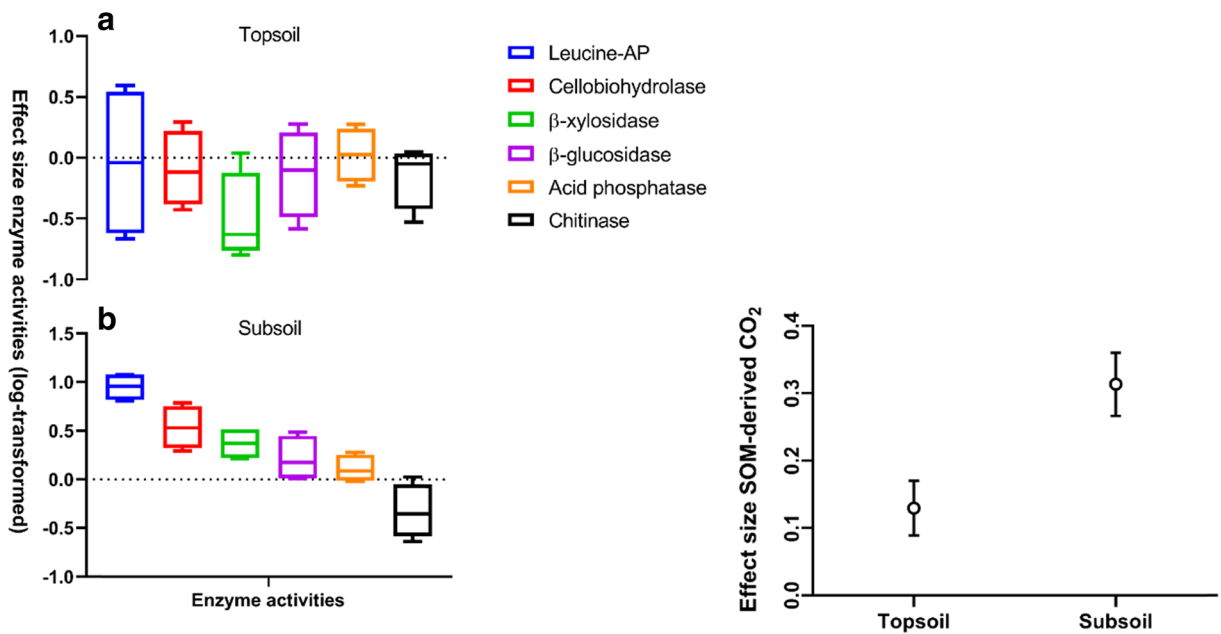


Fig. 6 Effect size of enzyme activities (left) over all plant densities for (a) topsoil and (b) subsoil are shown in the boxplot. Whiskers refer to 5 to 95% percentiles. The median is denoted by the line in the box. Effect size of SOM-derived CO₂ relative to

the bulk soil-derived CO₂ (right) over all plant densities for topsoil and subsoil treatments. All data was log-transformed before analysis

entered the soil (Lützwow et al. 2006; Pausch and Kuzyakov 2012). Subsoil SOM is often enriched in microbial-derived C compounds, as it is highly processed with time (Banfield et al. 2017; Tückmantel et al. 2017). In contrast, topsoil SOM primarily comprised of energy-rich plant material (Rumpel and Kögel-Knabner 2011). The subsoil plays a pronounced role in nutrient

mobilization and re-allocation across the whole profile (Kautz et al. 2013), and it provides an additional nutrient reservoir for future sustainable plant production (Eilers et al. 2012). Deep-growing roots supply extra access to N and P (Tarkalson et al. 1998) and C-limited subsoil organisms may get access to labile root-derived C compounds affecting SOM decomposition (Zhang et al. 2017). During drought or periods of nutritional deficiency in the topsoil, plants may increase their access to resources by subsoil rhizosphere priming, inferring considerable contribution to plant nutrition. Here, we demonstrated that subsoil properties encouraged shifts in the C allocation pattern and soil C dynamics in wheat.

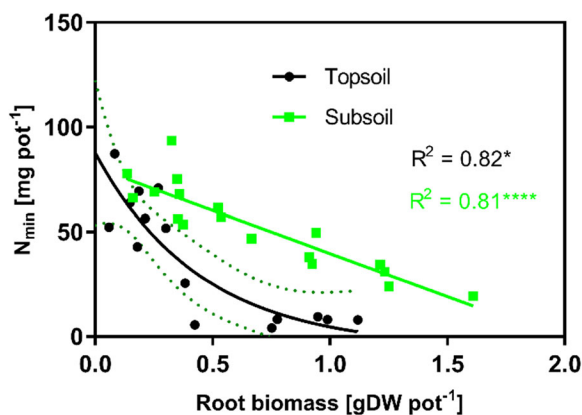


Fig. 7 Mineral nitrogen (N_{min}) in the topsoil decreased stronger with increasing root biomass compared with the subsoil. Mineral fertilizer addition treatment is excluded. Dashed line shows the 95% significance band. *****P* ≤ 0.0001; **P* ≤ 0.05

Enzyme properties in topsoil and subsoil

The stronger correlation between most enzyme activities and root biomass in the topsoil than in the subsoil (Fig. 2) indicated intensified production of extracellular enzymes by plants to acquire soil nutrients, likely induced by higher C supply due to increased photosynthesis of plants grown in topsoil. This results in strong competition or facilitation between individuals sharing resources under limited nutrient availability (Kuzyakov and Xu 2013). The change in *K_a* of acid phosphatases

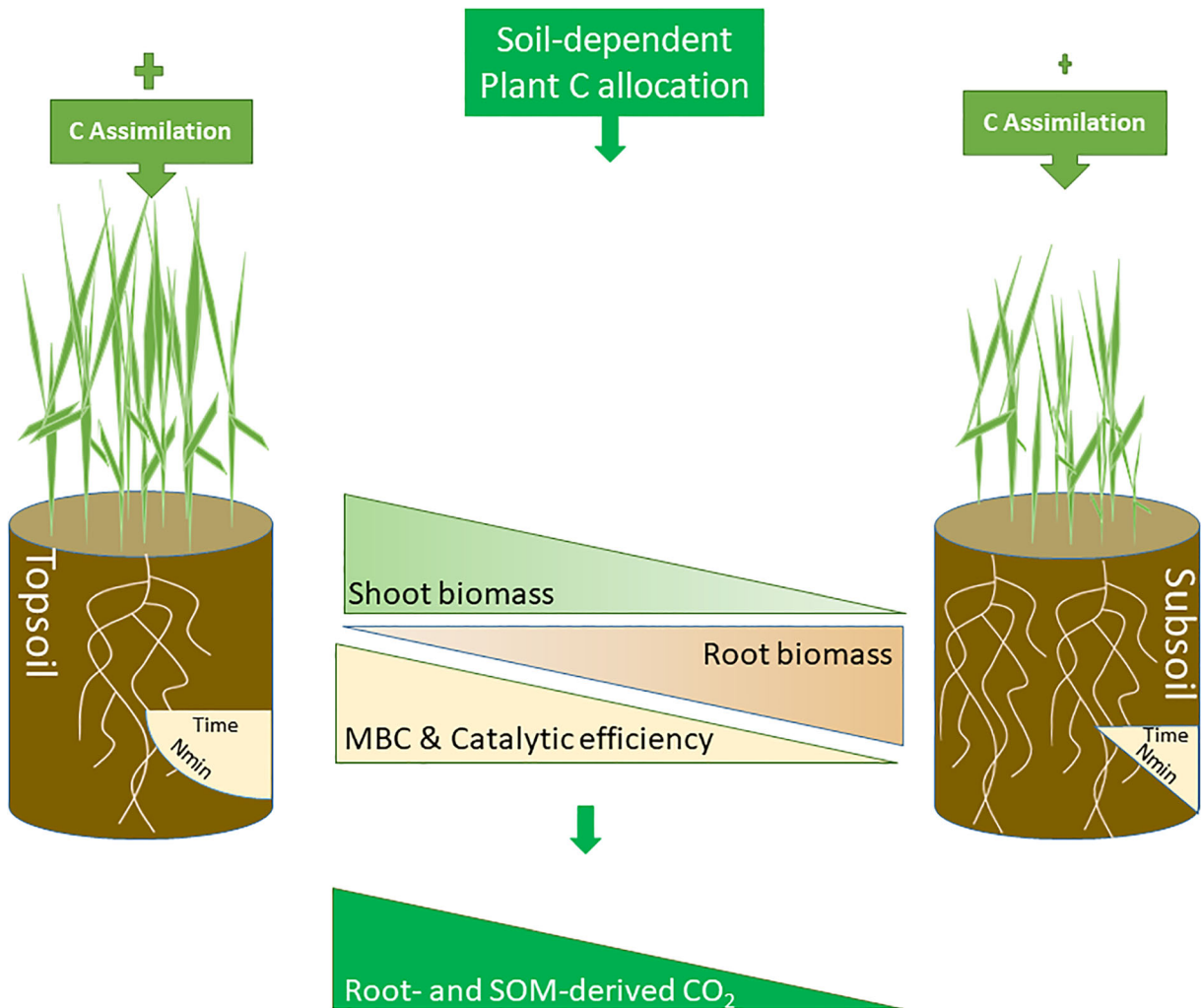


Fig. 8 Comparison of parameters: stronger assimilation of C, higher shoot, MBC, catalytic efficiency in topsoil than in subsoil

indicated lower P availability in the subsoil (T_{20}) (Supplementary Fig. 1), since V_{\max} was enhanced in both planted top- and subsoil (Fig. 5). As the enzyme affinity to the substrate shifted, catalytic efficiency of acid phosphatases was lower in planted topsoil. This shift was also demonstrated by the proportional enzyme activities of C- versus P-acquiring enzymes (Supplementary Fig. 4). The contrasting K_a of CE and BG between top- and subsoil (Supplementary Fig. 1), caused by the lower microbial biomass in the subsoil (Table 1), reflected the varying investments in extracellular enzyme hydrolysis of insoluble cellulose requiring endoglucanases, exoglucanases (cellobiohydrolases), and β -glucosidases working in synergy (Van Dyk and Pletschke 2012).

Plant and enzymatic responses on mineral N addition

The response of cereals to applications of N fertilizer has been shown to significantly increase the shoot to root ratio (Barraclough 1984). Here, this response was unique to the topsoil (Table 1). Poeplau et al. (2017) did not find any effect of N fertilization on the ratio, whereas Hirte et al. (2018) demonstrated that shoot to root ratios are regulated by fertilization intensity than by site. A strong non-linear negative relationship between N_{\min} and root biomass was found in the topsoil, whereas a linear decrease was observed for the subsoil (Fig. 7), which may be explained by much higher shoot biomass production in topsoil and more complete depletion of nutrients.

A recent study, mimicking root exudation by glucose addition, showed that N limitation largely controlled C turnover in the subsoil (Liang et al. 2019). Nitrogen addition did not have any effect on respiration (SOM-derived, root-derived CO₂ efflux) in the presence of plants (Fig. 3), contradicting several other studies (e.g., Ramirez et al. 2010). However, organic N cycling may decline due to a reduction in the activity of enzymes that target nitrogenous compounds (Allison et al. 2010), which was indicated by the slightly lower leucine-aminopeptidase activity when N was added irrespective of top- or subsoil (Supplementary Fig. 3).

Conclusion

The subsoil treatments had lower rhizosphere activity, greater root biomass, and lower shoot to root ratios than the topsoil treatments. This indicates a reduced allocation of C from shoot to rhizodeposition in the subsoil treatments. The presence of plants increased the rate of SOM decomposition in both the topsoil and subsoil treatments relative to the unplanted control. Root-derived CO₂ increased with increasing plant density, suggesting a close relationship with photosynthetic activity. Overall, plants grown in topsoil used higher amounts of C to supply rhizodeposition and respiration processes (indicated by root-derived CO₂) than plants grown in subsoil, thereby maintaining higher microbial activity in the rhizosphere. SOM decomposition tended to increase with plant density in both the topsoil and subsoil, which may indicate that the quantity of root exudates determines the impact on SOM decomposition. Enhanced subsoil sensitivity to C input demonstrated the importance of the rhizosphere inputs on microbial activity and SOM decomposition in deeper soil layers. Based on these results, we conclude that rhizosphere activity is mainly driven by aboveground C assimilation and allocation to rhizosphere processes.

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