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Research

Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity

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Summary

- Root exudation is a key plant function with a large influence on soil organic matter dynamics and plant–soil feedbacks in forest ecosystems. Yet despite its importance, the main ecological drivers of root exudation in mature forest trees remain to be identified.
- During two growing seasons, we analyzed the dependence of *in situ* collected root exudates on root morphology, soil chemistry and nutrient availability in six mature European beech (*Fagus sylvatica* L.) forests on a broad range of bedrock types.
- Root morphology was a major driver of root exudation across the nutrient availability gradient. A doubling of specific root length exponentially increased exudation rates of mature trees by *c.* 5-fold. Root exudation was also closely negatively related to soil pH and nitrogen (N) availability. At acidic and N-poor sites, where fungal biomass was reduced, exudation rates were *c.* 3-fold higher than at N- and base-richer sites and correlated negatively with the activity of enzymes degrading less bioavailable carbon (C) and N in the bulk soil.
- We conclude that root exudation increases on highly acidic, N-poor soils, in which fungal activity is reduced and a greater portion of the assimilated plant C is shifted to the external ecosystem C cycle.

Introduction

Root exudation is a pivotal process that determines rhizosphere functions and plant–soil relationships (Phillips *et al.*, 2011, 2012; Keiluweit *et al.*, 2015). Even though the specific rate of exuded carbon (C) can be low, its continuous release into the soil makes it a significant source of organic C. Root exudation represents a major portion of the assimilated carbohydrates of a plant and can consume up to one-third of the total photosynthates (Liese *et al.*, 2018). Root exudation of labile C provides soil microorganisms in the rhizosphere with an easily accessible and important energy source.

Soil microorganisms convert soil organic matter (SOM) into bioavailable forms (Read & Perez-Moreno, 2003). Yet at any time, a large portion of the microbial community is energy-limited and functionally inactive (Prosser *et al.*, 2007) and their enzyme production is regulated by economic rules (Allison & Vitousek, 2005). Accordingly, the microbial production of hydrolases (which depolymerize cellulose and chitin into bioavailable forms) is tied closely to substrate availability (i.e. SOM or total C content) and pH optima, both within and across biomes (Sinsabaugh *et al.*, 2008; Talbot *et al.*, 2013). By contrast, the effect of nitrogen (N) availability on hydrolytic activity remains inconclusive. Different studies have shown that hydrolases are either suppressed (Burns *et al.*, 2013), unaffected (Zeglin *et al.*, 2007) or stimulated (DeForest *et al.*, 2004; Allison *et al.*, 2008) by N deficiency, in dependence on the relative amount of (mycorrhizal) fungi as the main producers of chitinases and polysaccharide hydrolases (Baldrian, 2008; Billings & Ziegler, 2008). These conflicting results among studies probably reflect the effect of colimitation of enzyme production by bioavailable C. Soil N deficiency can decrease *N*-acetyl-*β*-glucosaminidase (NAG) activity when either the microbial community is shifted to taxa with inherently lower N demand or the bioavailability of C is increasingly limiting enzyme production (Blagodatskaya & Kuzyakov, 2008; Talbot *et al.*, 2013). However, N limitation can also increase NAG activity when soil microorganisms are less C-demanding or are released from colimitation by labile C inputs, such as from root exudation (Allison & Vitousek, 2005; Meier *et al.*, 2015).

Nutrient deficiency affects the growth and vitality of the fine root system. It has been demonstrated that it induces variable root responses, among them a larger specific root length (SRL), a smaller root diameter and greater root biomass allocation (Kramer-Walter & Laughlin, 2017; Li *et al.*, 2019), and longer root lifespan (Eissenstat *et al.*, 2000; Van der Krift & Berendse, 2002). Yet the implications of these root responses for the

quantity of root exudates are poorly understood. Previous research has often focused on the consequences of root exudation for the resource uptake of plants from the rhizosphere (e.g. Keiluweit *et al.*, 2015; Canarini *et al.*, 2019). It was demonstrated that elevated exudation of labile C causes a relative increase in microbial activity and in native C mineralization in the rhizosphere (defined as the 'microbial priming effect'; Kuzyakov, 2010). This increase in SOM mineralization can be sufficiently large to boost nutrient availability in the rhizosphere to the extent that N limitation is delayed (Phillips *et al.*, 2011). Despite this general knowledge on the influence of root exudation on rhizosphere processes, the controls on the quantity of exuded C are less well understood.

The quantity and chemical composition of root exudates are thought to be influenced by root architecture, environmental conditions, and the presence of beneficial or pathogenic soil microbes (Neumann & Römheld, 2007). It has been suggested that root exudation is strongly influenced by the amount of available photosynthates and thus depends on controlling factors such as photosynthetically active radiation, atmospheric CO_2 concentration, N availability and soil moisture (Kuzyakov & Cheng, 2001; Kuzyakov, 2002; Nakayama & Tateno, 2018). However, several studies have shown that root exudation of trees also increases with SRL (Tückmantel *et al.*, 2017), deficiency of nutrient elements such as phosphorus (P) and N (Ward *et al.*, 2011; Yin *et al.*, 2014), temperature (Boone *et al.*, 1998), drought stress (Preece *et al.*, 2018) and the type of fungi colonizing the roots (Meier *et al.*, 2013). The net effect of different factors influencing root exudation is difficult to predict, because, for example, fungal sheath formation by mycorrhizae may hinder root exudation (Neumann & Römheld, 2007; Meier *et al.*, 2013), while at the same time mycorrhizal fungal secretion of organic compounds can increase exudation from mycorrhizal roots (Lioussanne *et al.*, 2008; Frey, 2019). The relative importance of different intrinsic, biotic and abiotic factors as determinants of root exudation in a natural and complex forest setting thus remains unclear.

In this study, we investigated fine root morphology and fine root exudation in mature European beech forests across a broad range of bedrock types in two growing seasons. The aim was to detect adaptive responses of the root exudation rate of beech at sites with largely different soil chemistry and nutrient availability. We hypothesized that the less bioavailable organic compounds in acidic forest soils decrease microbial activity and N supply, while the SRL of beech roots increases, both of which result in elevated root exudation rates compared to less acidic, base-richer soils.

Materials and Methods

Study sites, geology and climate

Root exudation was analyzed in six mature European beech forest stands, which differed in bedrock type (Table 1). Important criteria for study site selection were comparability of climate, relief, tree species (single-species European beech stands; homogeneous stand structure with closed canopy; comparable stem density) and time (soil development since the Holocene; beech stands of similar age). This allowed us to investigate the role of the fifth ecosystem state factor (i.e. parent material) on soil nutrient status and root exudation, adopting the concept of 'ecosystem state factors' of Jenny (1941). The study sites were chosen in a restricted area of central Germany (southern Lower Saxony) at a maximum distance of 128 km. All sites were located below 500 m above sea level mostly in the colline and submontane belts at level to slightly inclined terrain. The stands were selected on a soil chemical gradient from extremely acidic, sandy soils to base-rich, calcareous soils. Sandy glacial deposits of the penultimate Ice Age (Saalian) cover the north of the study region, whereas the south represents a small-scale mosaic of various Mesozoic and Cenozoic bedrock types. The chosen bedrock types range from the Triassic to the Quaternary and thus span an epoch of *c.* 240 million years. They include limestone, basalt, loess, sandstone, sand and glacial deposits. The soils were mainly Cambisols in a variety of subtypes as well as Luvisol on loess (classified according to IUSS Working Group WRB, 2015; Table 2). None of the sites was influenced by groundwater. The study region has a temperate suboceanic climate with mean annual temperatures of 7.1–8.7°C and mean annual precipitation of 709–902 mm yr^{-1} (Table 1).

Root exudate collection

Exudates were collected in three sampling campaigns during the growing seasons 2014 and 2015 (i.e. in May 2014, August 2014 and June 2015) using a culture-based cuvette system (cf. Phillips *et al.*, 2008; Ostonen *et al.*, 2020). Three fine root systems each were sampled in three soil pits per study site ($n = 9$ samples). Fine root samples were taken at a distance of at least 3 m to the nearest mature beech tree. Terminal fine roots attached to a mature beech tree were extracted from the mineral topsoil below the organic layer with extreme caution. Soil particles adhering to the root system were carefully removed with deionized water and fine forceps to maintain the integrity of the root. The whole process took *c.* 30–60 min per root system. Any root system that appeared damaged from this process upon visual inspection was excluded from further analyses. Subsequently, roots were placed overnight in moist, sandy soil to allow recovery from the excavation process. On the next day, the living root systems were placed into root cuvettes filled with sterile glass beads moistened with C-free nutrient solution (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.15 mM $MgSO_4$, 0.3 mM $CaCl_2$). The enclosed fine root strands had an average diameter of *c.* 0.4 mm and were *c.* 15 cm long. In this solution culture system, the glass beads provided the mechanical impedance and porosity of soils but in a matrix free of C. Sterile cuvettes with glass beads and nutrient solution (i.e. no roots) were included as controls. Roots could recover and equilibrate in the cuvette environment for 48 h before being flushed with dilute nutrient solution using a low-pressure vacuum. New C- and N-free nutrient solution was added and equilibrated for another 21 h. We collected these trap solutions containing exudates from each cuvette, filtered them

Table 1 Parent material, geological epoch, location, altitude, mean annual precipitation and temperature, forest association, and tree age of European beech forests on six different bedrock types.

Parent material	Epoch	Longitude (E)	Latitude (N)	Altitude (m above sea level)	Precipitation (mm)	Temperature (°C)	Association	Tree age (yr)
Limestone	l MU	10°02′	51°32′	410	881	7.1	HF	166
Basalt	t B	09°45′	51°28′	470	902	7.1	GF	153
Loess	pl L	10°14′	51°34′	200	709	8.1	GF	95
Sandstone	m BU	10°03′	51°34′	295	772	7.7	LF	133
Sand	t S	09°41′	51°26′	270	761	8.1	LF	118
Glacial deposit	pl FS	09°20′	52°14′	100	718	8.7	FQ	100

Geological epoch: l MU, Lower Muschelkalk; m BU, Middle Bunter; pl FS, Pleistocene fluvioglacial sand, penultimate Ice Age (Saalian); pl L, Pleistocene loess, last Ice Age (Weichselian); t B, Tertiary basalt; t S, Tertiary sand. Association: GF, Galio odorati-Fagetum; HF, Hordelymo-Fagetum; LF, Luzulo-Fagetum; FQ, Fago-Quercetum (Luzulo-Fagetum, lowland type).

Table 2 Humus form, organic layer depth, soil type, pH, nutrient availability and extracellular enzyme activities in the topsoil of European beech forests on six different bedrock types.

		Limestone	Basalt	Loess	Sandstone	Sand	Glacial deposit
Humus form		vm	m	lm	lm	hm	mm
Organic layer depth (mm)	Mean	18	37	20	19	44	35
Soil type		cCa	eCa	hLu	dCa	hCa	dCa
pH ($CaCl_2$)	Mean	4.0^{A}	3.7^{A}	3.6^{AB}	3.9^{A}	3.7^{A}	3.4^{B}
	+SE	0.03	0.03	0.01	0.08	0.07	0.05
	−SE	0.03	0.03	0.01	0.06	0.06	0.04
SOC (%)	Mean	2.2^{b}	3.6^{a}	1.0^{d}	1.4^{cd}	1.6^{c}	1.2^{cd}
	SE	0.2	0.2	0.1	0.1	0.1	0.05
SON (g N kg^{-1})	Mean	1.67^{B}	2.64^{A}	0.77^{CD}	0.82^{C}	0.78^{C}	0.45^{D}
	SE	0.11	0.13	0.05	0.06	0.02	0.03
C : N (g g^{-1})	Mean	13.2^{c}	14.1^{bc}	12.8^{c}	17.4^{ab}	20.8^{a}	18.7^{bc}
	SE	0.5	0.2	0.1	0.3	0.1	0.5
Microbial activity							
(Hemi-)Cellulase activity (μg C g_{soil}^{-1} h^{-1})	Mean	0.53^{BC}	2.23^{A}	1.46^{AB}	0.80^{BCD}	0.49^{C}	0.14^{D}
	SE	0.22	0.34	0.84	0.42	0.03	0.01
NAG activity (μg N g_{soil}^{-1} h^{-1})	Mean	0.58^{abc}	1.70^{a}	1.33^{abc}	0.69^{abc}	1.04^{b}	0.43^{c}
	SE	0.40	0.21	0.99	0.39	0.15	0.04
AP activity (μg P g_{soil}^{-1} h^{-1})	Mean	9.9^{B}	75.2^{A}	20.8^{B}	11.2^{B}	12.3^{B}	8.7^{B}
	SE	5.7	13.7	9.5	2.6	1.0	0.8
Fungal biomass (μg C g^{-1})	Mean	n/d	1721	151	n/d	28^{1}	0.18^{2}

Humus form: hm, hemimor; lm, leptomoder; m, mullmoder; mm, mormoder; vm, vermimull. Soil type (classification according to IUSS Working Group WRB, 2015): c, chromic; Ca, Cambisol; d, dystric; e, eutric; h, haplic; Lu, Luvisol. Different upper-case or lower-case letters in a row indicate significant differences among bedrock types ($n = 4$ pits per substrate; glacial deposits, $n = 8$ pits). AP, alkaline phosphatase; NAG, *N*-acetyl-*β*-glucosaminidase; n/d, not determined; SOC, soil organic carbon; SON, soil organic nitrogen.
[1]Based on the quantity of microbial biomarkers (Angst *et al.*, 2018).
[2]Based on $\delta^{13}C$ phospholipid fatty acid analyses (Preusser *et al.*, 2017).

through sterile syringe filters (pore size 0.7 μm, Whatman glass microfiber filters, grade GF/F; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and immediately froze the filtered solution at −20°C. Trap solutions were analyzed for dissolved organic C on a total organic C analyzer (Shimadzu TOC-L CPH/CPN; Shimadzu Scientific Instruments, Duisburg, Germany). Net mass-specific exudation rates (gross root exudation minus reabsorption and microbial consumption) were calculated as the total amount of C flushed from each root system over the incubation period divided by the total root mass in the cuvette (μg C g_{root}^{-1} h^{-1}). The annual C flux from exudation (in g C m^{-2} yr^{-1}) was estimated by multiplying the average mass-specific exudation flux for each individual soil pit with the average biomass of finest roots <1 mm diameter in the organic layer and top mineral soil (0–10 cm) and multiplying daily exudation rates by the average length of the growing season with a positive C balance for European beech in the northern part of Central Germany (186 d; following Schulze, 1970). We assumed an increase in growing season length in Central Europe since the 1970s by at least 10 d with climate change (cf. Menzel & Fabian, 1999; Jeong *et al.*, 2011). The average biomass of finest roots <1 mm was estimated from the fine root biomass measured for the organic layer and top mineral soil (see subchapter on 'Root morphology and root biomass' section below) and the distribution of beech fine roots into diameter subclasses according to Montagnoli *et al.* (2018) (59% of fine roots in <1 mm class).

Root morphology and root biomass

After root exudate collection, the sampled fine roots were clipped from the tree, immediately transported to the laboratory and stored at 4°C until processing. Fine root morphology was analyzed for all fine root samples by optical surface area measurement with a flat-bed scanner and the program WinRHIZO (Régent Instruments, Québec, QC, Canada). Subsequently, root biomass was determined by drying (48 h, 70°C) and weighing. SRL ($m\,g^{-1}$) was calculated from these measurements.

Fine root biomass in the organic layer and top mineral soil (0–10 cm soil depth) were investigated by soil coring at all study sites in June 2013 (glacial deposits) and May 2014 (all other bedrock types). Each of the six soil samples per forest stand were taken with a soil corer (3.5 cm in diameter) from the uppermost 10 cm of the soil profile (including the organic layer) at random coordinates within a 30 × 30 m plot and divided into two subsamples (organic layer and 0–10 cm). The material was immediately transported to the laboratory and stored at 4°C for no longer than 4 wk. Only beech fine roots <2 mm in diameter were considered for analysis. Fine roots were picked out by hand and sorted into live and dead fine root mass under a stereomicroscope (×40). Criteria for assessing root vitality were the color and structure of the root surface, root elasticity and turgescence, branching structure, and the degree of cohesion of cortex, periderm and stele (for criteria, see Persson, 1978; Meier & Leuschner, 2008). Finest root biomass (<1 mm in diameter; see 'Root exudate collection' above) was expressed as profile totals (organic layer and 0–10 cm of mineral soil; in $g\,m^{-2}$).

Soil chemical analyses

In May 2014, soil sampling was performed at every study site in one randomly placed grid frame of 90 × 135 cm, which was equally divided into six grid cells of 45 × 45 cm. We excavated small soil pits to enable horizontal coring with a steel ring (8.5 cm in diameter, 6 cm in height). Soil samples were taken horizontally at each corner of the grid cells at 5 cm soil depth ($n = 12$ soil samples). Field-moist mineral soil samples were analyzed for pH ($CaCl_2$) in 0.01 M $CaCl_2$ (1 : 2.5, w/v) after 1 h of equilibration. Plant-available P according to Bowman & Cole (1978) was extracted by using resin bags (anion exchange gel, Dowex 1 x 8-50; Dow Water & Process Solutions, Edina, MN, USA) that were placed for 16 h in a solution of 1 g of soil material suspended in 30 ml water (Sibbesen, 1977). Phosphorus was re-exchanged by NaCl and NaOH solutions and analyzed by a color reaction with 5 mM hexaammonium heptamolybdate (Murphy & Riley, 1962) and photometric measurement at 712 nm (Libra S 21 spectrophotometer; Biochrom, Cambridge, UK). Total C and N in the mineral soil were determined in samples dried at 60°C (48 h) with an elemental analyzer (vario El Cube; Elementar Analysesysteme GmbH, Hanau, Germany). The gravimetric soil water content (% SWC, w/w) was determined by drying (110°C, 48 h) soil samples to constant weight and weighing soil sample mass before and after drying.

Microbial biomass and soil enzyme activities

The chloroform fumigation direct extraction (CFE)-method (Vance *et al.*, 1987) was used to determine microbial biomass C (C_{mic}). Nonpurgeable organic C and total N were measured using a TOC-TNb Analyzer (Multi-N/C 2100S; Analytik Jena, Jena, Germany). Because only visible roots were removed before fumigation of the samples, a slight fine root-derived C contribution to chloroform-labile C cannot be fully excluded (Mueller *et al.*, 1992). Microbial C was calculated using a k_{EC} factor of 0.45 (Joergensen, 1996) and was given as $\mu g\,C_{mic}\,g_{soil}^{-1}$.

A subsample of soil was stored at −20°C before enzyme analysis. Activities of six extracellular enzymes involved in the decomposition of C-, N- and P-containing compounds were assayed (Marx *et al.*, 2001). Activities of enzymes were measured with methylumbelliferyl (MUF)-labeled substrates. The six enzymes can be functionally grouped based on their ability to decompose hemicelluloses and celluloses (α-glucosidase, AG; β-glucosidase, BG; β-xylosidase, BX; β-cellobiosidase, BC) or depolymerize organic N (NAG) or P (alkaline phosphatase, AP). Enzyme activities were analyzed using a fluorescence microplate reader (TECAN infinite 200; TECAN Group, Männedorf, Switzerland) with 360 nm excitation and 465 nm emission filters. Enzyme activities were expressed in units of mg substrate cleaved $g_{soil}^{-1}\,h^{-1}$ or μg substrate cleaved $g^{-1}\,C_{mic}\,h^{-1}$.

Statistical analyses

Statistical analyses were conducted with the package SAS version 9.3 (Statistical Analyses System; SAS Institute Inc., Cary, NC, USA). Significance was determined at $P \leq 0.05$. The probability of a fit to a normal distribution was tested using a Shapiro–Wilk test. Study site means were compared by one-way ANOVA or by one-way Kruskal–Wallis single factor analyses of variance and nonparametric multiple comparison tests after Wilcoxon to analyze the differences between soil depths or sampling dates. Mixed variance–covariance models for fixed and random effects with the variables substrate and sampling date were calculated to test for significant effects. Data likelihood was maximized to estimate the model parameters. Pits were included as random effects. We conducted multiple regression analyses with backward variable elimination to test for significant independent predictors of root exudation rates and the annual C flux from root exudation. At each elimination step, the variable showing the smallest contribution to a model was deleted until all the variables remaining in the model produced significant F statistics. Multicollinearity among variables was diagnosed when the significance of the t tests for all individual slopes differed from the F test of the model, pairs of predictor variables were highly correlated and collinearity diagnostics were critical.

Results

Bedrock effects on root exudation in the topsoil

On average, topsoil roots exuded $29 \pm 7\,\mu g\,C\,g^{-1}\,h^{-1}$ across the different bedrock types (Fig. 1a). The lowest mass-specific

exudation rates were observed on loess and basalt (16–19 μg C g^{-1} h^{-1}), intermediate values on sand (31 μg C g^{-1} h^{-1}) and the significantly highest exudation rates in the topsoil on glacial deposits (65 μg C g^{-1} h^{-1}). The finest root biomass (<1 mm) was lowest in the topsoil on basalt and loess substrates (53–62 g m^{-2} in the organic layer and 0–10 cm of mineral soil; Supporting Information Table S1). Intermediate finest root biomass was observed on glacial deposits and limestone (64–74 g m^{-2}), whereas the topsoil on sandstone and sand contained the significantly highest finest root biomass (103–110 g m^{-2}). This resulted in a relatively small annual C flux from root exudation in the topsoil of the basalt and loess sites (4–5 g C m^{-2} yr^{-1}) and intermediate or high C flux on the sandy substrates (intermediate: sandstone, 9 g C m^{-2} yr^{-1}; high: sand and glacial deposit, 15–16 g C m^{-2} yr^{-1}; Fig. 1b). Correspondingly, the annual C flux from root exudation increased by *c.* 4-fold from the carbonaceous substrates to the sandy sediments.

The effect of the geological substrate on the root exudation rate was highly significant and stronger than that of the sampling date (Table 3). Root exudation differed between the two study years: the mass-specific root exudation rate averaged at 31 ± 12 and 35 ± 9 μg C g^{-1} h^{-1} in May and August 2014, respectively, but decreased to 22 ± 5 μg C g^{-1} h^{-1} in June 2015 (Fig. S1). Across the sampling dates, mass-specific root exudation rates were always highest on glacial deposits and low on basalt and loess. However, the specific rank order of root exudation rates on the carbonaceous substrates was more variable across sampling dates than the top rank for the glacial deposits. Root exudation rates on limestone showed high variability across the sampling dates (coefficient of variation: 89%). Generally, an increase in mean annual temperature by 1°C at a study site increased the annual C flux from root exudation by 6 g C m^{-2} yr^{-1} (Fig. 2e).

Relationship between root morphology and root exudation

Roughly in parallel to the root exudation rates, SRL of the sampled root systems increased from low values on loess and basalt (13–17 m g^{-1}) to intermediate values on sand (21 ± 1 m g^{-1}) and high values on glacial deposits (23 ± 2 m g^{-1}; Fig. 3). This increase in SRL was related to a concomitant decrease in root diameter ($P < 0.001$; Fig. S2). Next to the effect by the substrate, the sampling date had a strong influence on SRL ($P < 0.001$; Table 3). Across the geological substrates, SRL was comparably low in May 2014 (15 ± 1 m g^{-1}) and increased towards June 2015 (23 ± 2 m g^{-1}; Fig. S3). SRL had a positive influence on root exudation. With an increase in SRL from 12 to 24 m g^{-1}, mass-specific root exudation rate increased exponentially from 87 to 397 mg C g^{-1} yr^{-1} ($P < 0.001$; Fig. 2a) and the annual C flux from 3.8 to 19 g C m^{-2} yr^{-1} ($P = 0.004$; Fig. S2).

Relationship between nutrient availability and root exudation

The investigated beech forests grew on a range of soil types spanning from eutric Cambisols and haplic Luvisols on basalt and loess substrates to dystric Cambisols on sandstones and glacial deposits (Table 2). The humus form ranged from thin vermimull on limestone to thick mormoder and hemimor on glacial deposits and sand, respectively. The soil pH of the mineral topsoil (0–10 cm) varied only little but was significantly lower in soil on glacial deposits (pH in $CaCl_2$ 3.4; significant difference to that on sand, sandstone, basalt and limestone, pH 3.7–4.0). The root exudation rate increased three-fold with this doubling in soil acidity from 0.2 to 0.4 mM H^+ (i.e. with a decrease in pH from 3.7 to 3.4; $P = 0.004$; Fig. 2b).

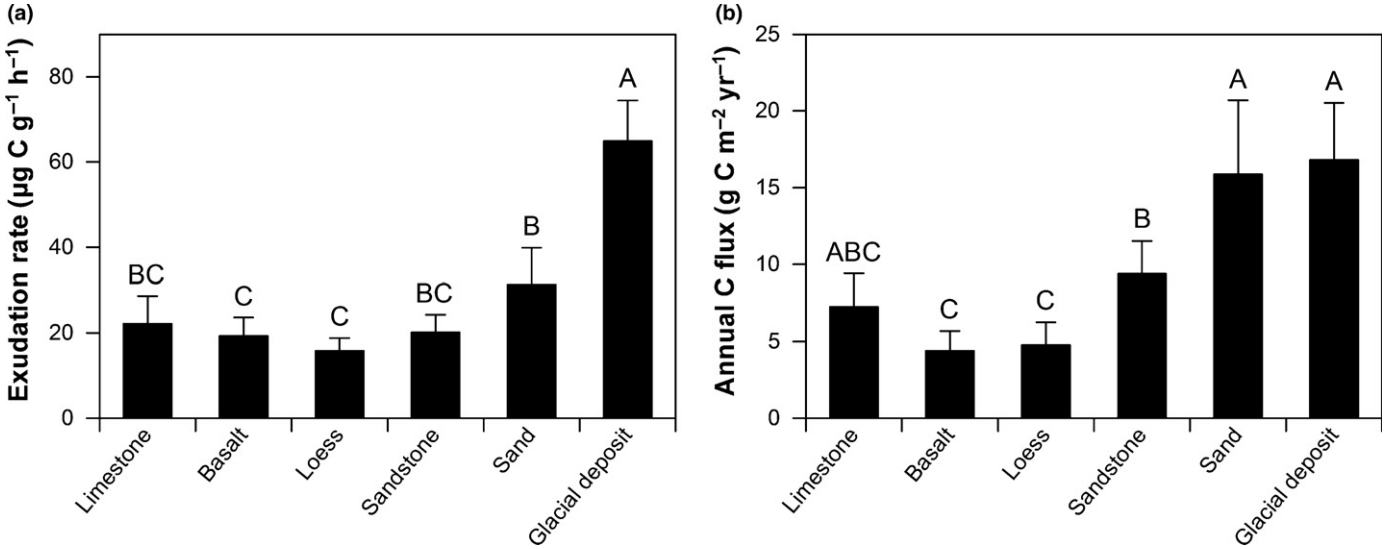

Fig. 1 Means + SE of (a) root exudation rates and (b) estimated annual carbon (C) flux from root exudation in the topsoil of European beech forests on six different bedrock types ($n = 3$ pits and $n = 3$ dates per substrate). Different upper-case letters indicate significant differences between the substrates. For comparability among substrates, the annual C flux from root exudation refers to the organic layer and mineral topsoil (0–10 cm soil depth). The annual C flux was calculated by multiplying average exudation rate with the amount of finest root biomass (<1 mm in diameter) in the organic layer and 0–10 cm layer.

Table 3 Mixed effects models on the influence of geological substrate (Substrate) and sampling date (Date) on root exudation and specific root length (SRL) in the topsoil of European beech forests located on six different bedrock types.

	Root exudation (μg C g^{-1} h^{-1})		SRL (m g^{-1})	
	F	*P*	*F*	*P*
Substrate	17.5	<0.001	10.9	<0.001
Date	4.2	0.02	31.8	<0.001
Substrate × date	3.8	0.002	2.3	0.03

Soil pits were included as random effects ($n = 3$ replicates per substrate and sampling date).

Soil organic carbon (SOC) and soil organic nitrogen (SON) in the topsoil were high on basalt (36 g C_{org} kg^{-1}, 2.6 g N_{org} kg^{-1}), intermediate on limestone (22 g C_{org} kg^{-1}, 1.7 g N_{org} kg^{-1}) and lowest on the other substrates (10–16 g C_{org} kg^{-1}, 0.5–0.8 g N_{org} kg^{-1}; Table 2). The mineral topsoil on the glacial deposits was distinguished by a particularly low SON content (0.5 g N_{org} kg^{-1}). The C : N ratio of the mineral topsoil was low on loess, limestone and basalt (13–14 g g^{-1}) but increased towards the site on sand (21 g g^{-1}). This 1.6-fold increase in the soil C : N ratio was related to a 2.6-fold increase in the annual C flux from root exudation ($P < 0.001$; Fig. 2f).

Soil biotic influences on root exudation

The mineral topsoil on basalt and limestone had the significantly highest amount of microbial biomass (182–218 μg C_{mic} g^{-1}; Fig. 4a), which was *c.* 4.5 times higher than on all other bedrocks. Microbial biomass was especially low at the sand and sandstone sites (22–36 μg C_{mic} g^{-1}). The activities of (hemi-)cellulase and NAG in the bulk soil were high on basalt and loess (basalt: 2.2 μg C g_{soil}^{-1} h^{-1}, 1.7 μg N g_{soil}^{-1} h^{-1}; loess: 1.5 μg C g_{soil}^{-1} h^{-1}, 1.3 μg N g_{soil}^{-1} h^{-1}) and strongly reduced in the bulk soil on the glacial deposits (0.1 μg C g_{soil}^{-1} h^{-1}, 0.4 μg N g_{soil}^{-1} h^{-1}; Table 2). The activities of (hemi-)cellulase and NAG were related negatively to the root exudation rate, which exponentially increased with a decrease in the bulk soil microbial activity ($P = 0.001$ and 0.01, respectively; Fig. 2c,d). When considering the specific NAG activity of the microbial biomass (i.e. the production of extracellular enzymes per microbial biomass), the pattern was almost opposite across bedrock types to that found for the enzyme activity per bulk soil mass. Specific microbial activity was high on sand, sandstone and loess (30–64 ng N g^{-1} C_{mic} h^{-1}), but strongly reduced on limestone (3 ng N g^{-1} C_{mic} h^{-1}; Fig. 4b). In contrast to (hemi-)cellulase and NAG, the activity of AP in bulk soil (or the amount of plant-available P) had no significant relationship to the root exudation rate (Fig. S4).

Multiple regression analyses comparing intrinsic, biotic and abiotic influences on root exudation

Our multiple regression analyses revealed that the most important predictor of the root exudation rate was soil pH, that is the root's soil chemical environment ($P < 0.001$; Table 4), followed by root morphology, which manifests in a positive relationship with SRL ($P < 0.001$). A minor predictor of the root exudation rate was the activity of NAG in the bulk soil with high values on SON-rich basalt and low values on SON-poor glacial deposits. NAG activity in the bulk soil was negatively related to root exudation rates ($P = 0.02$).

When considering the annual C flux from root exudation as the product of mass-specific exudation rate and finest root biomass, the importance of intrinsic influences on root exudation is greater. The most important predictor of the annual C flux was SRL ($P < 0.001$), followed by mean annual temperature at the study site ($P = 0.02$). By contrast, nutrient availability did not significantly influence the annual C flux from root exudation according to the multiple regression analysis.

Discussion

Understanding the response of root exudation of forest trees to variation in abiotic and biotic soil conditions will increase our capacity to predict global change effects on soil C stocks, SOM decomposition and C cycling. Most previous studies have focused on the consequences of root exudation for rhizosphere processes (e.g. Yin *et al.*, 2014; Keiluweit *et al.*, 2015; Sasse *et al.*, 2018), while, surprisingly, much less research has been directed toward the factors controlling the amount of C exuded by tree root systems. It has been shown that the root exudation of trees increases at elevated temperatures and atmospheric CO_2 concentrations, when N availability was an additional limiting factor (Phillips *et al.*, 2011; Yin *et al.*, 2013). Here, we demonstrate that root exudation of mature beech trees is also closely related to root morphology and soil acidity across a broad gradient of bedrock types and nutrient availabilities.

Root morphology as a driver of root exudation across different bedrock types

In our field study in European beech forests, root morphology exerted a major influence on C loss with root exudation. Other studies have pointed to the role of root morphology for exudation by showing that exudation rates of grasses are higher when root systems are on average thinner and have more root tips (Paterson & Sim, 2000; Darwent *et al.*, 2003). It appears that the relative abundance of young root segments with very low diameters is controlling exudation rates (Groleau-Renaud *et al.*, 1998). SRL (i.e. the length of absorptive root tissue deployed per unit biomass invested) can serve as a measure of the proportion of very fine roots in a root system. Root systems with a high SRL have on average thinner roots (Ma *et al.*, 2018), a lower cortex : stele ratio (Kong *et al.*, 2014), and lower arbuscular or ectomycorrhizal fungal colonization (Kong *et al.*, 2014; Brundrett & Tedersoo, 2018), because their smaller diameter provides less cortex habitat for mycorrhizal fungal symbionts (Guo *et al.*, 2008). In thinner roots, the C costs of root construction and of the mycorrhizal symbiosis are reduced and more C may be available for root exudation. In accordance with this, De Vries *et al.* (2016) speculated that root exudation is positively linked to SRL. Our

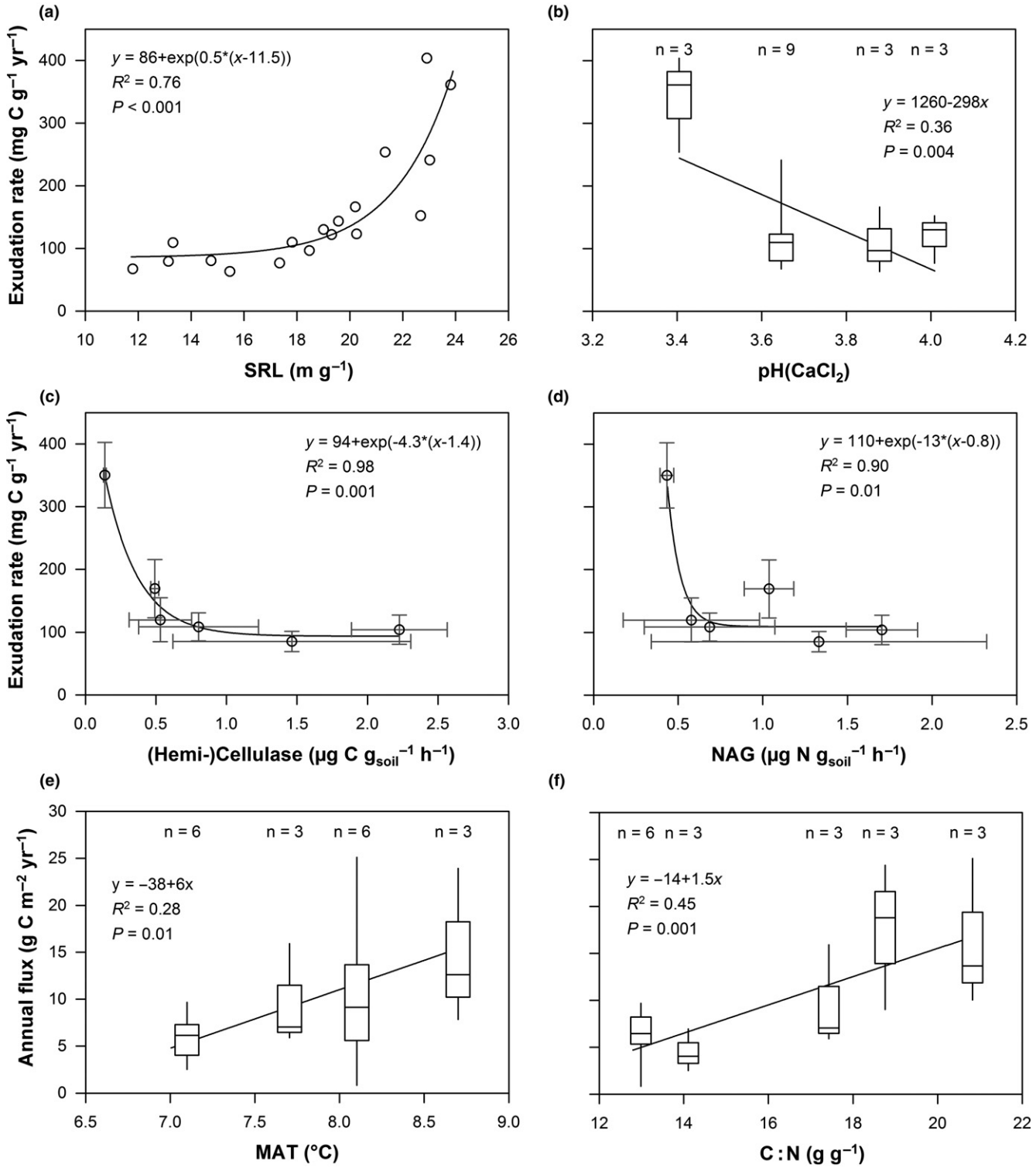

Fig. 2 Relationship of root exudation with (a) specific root length (SRL), (b) pH ($CaCl_2$), (c) (hemi-)cellulase (α-glucosidase + β-xylosidase + β-cellobiosidase) activity, (d) *N*-acetyl-*β*-glucosaminidase (NAG) activity, (e) mean annual temperature (MAT) and (f) soil carbon : nitrogen (C : N) in the topsoil of European beech forests on six different bedrock types. Shown are pit means + SE for exudation and SRL ($n = 3$ samples and $n = 3$ dates per substrate) and site means + SE for enzyme activities and C : N ($n = 4$ pits per substrate). Box plots depict the median (band), upper and lower quartile (box), and the upper and lower extremum (whiskers) of all exudation values at a site-specific environmental condition. Root exudation and SRL were sampled from May 2014 to June 2015; enzyme activities and C : N were sampled in June 2013 (glacial deposits) and May 2014 (all other substrates). The annual C flux was calculated by multiplying average exudation rate with the amount of finest root biomass (<1 mm in diameter) in the organic layer and 0–10 cm layer.

study shows that the relationship is in fact an exponential one, with a steep increase in exudation at SRL values greater than *c.* 20 m g^{-1} (when root diameter decreases below 0.43 mm). SRL was a major driver of root exudation across geological bedrock types, both when specific rates and the total net C release of the root system were considered. Yet more closely, there were two different causes for a higher estimated annual C flux from root exudation in acidic beech forests as compared to the other geological substrates. It was either due to very high finest root biomass (FRB) and higher specific exudation rates (on sand), or due to very high specific exudation rates and higher SRL (on glacial deposits). This difference in the putative causation of higher annual C exudation on the two sandy sites is apparently related to the doubling of the proton concentration in the soil solution on glacial deposits, which was shown to reduce mycorrhizal fungal colonization rates (St Clair & Lynch, 2005; Carrino-Kyker *et al.*, 2016; Leberecht *et al.*, 2016). It seems that both – a relative increase in the abundance of root segments with high exudation, and an increase in specific exudation activity in environments where mycorrhizal fungal colonization presumably is reduced – can play a role in the exudation response of European beech forests to acidic soil conditions.

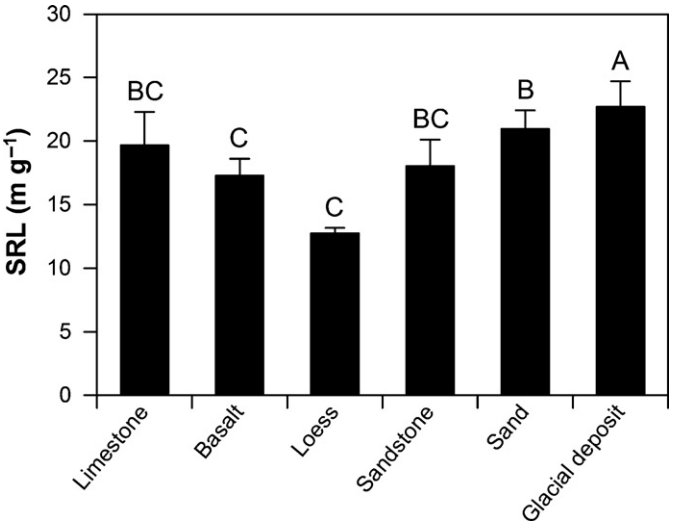

Fig. 3 Means + SE of specific root length (SRL) in the topsoil of European beech forests on six different bedrock types ($n = 3$ pits and $n = 3$ dates per substrate). Different upper-case letters indicate significant differences between the substrates.

Root exudation increases with soil acidity and nitrogen deficiency

Soil acidity was associated with elevated root exudation rates in the investigated European beech forests. The soil pH represents an umbrella characteristic of the chemical properties and nutrient availability in the soil. A decrease in soil pH induces changes in many factors that influence plant growth: the availability of the major nutrients N and P and of base cations is reduced, aluminum concentrations increase, and soil fungi may increasingly replace bacteria as the main agents of SOM decomposition (Rousk *et al.*, 2009). These different factors may have opposing effects on root exudation. In a study with soybean, root exudation was suppressed by a decrease in pH, but increased with an increase in aluminum phosphates in acidic soil (Liang *et al.*, 2013). Enhanced root exudation of organic acids in acid soil can counteract aluminum toxicity by complexion, which increases the availability of major nutrients (Ohta & Hiura, 2016).

The controlled release of root exudates in response to environmental stimuli is probably a major mechanism that allows plants to respond to (temporally) unfavorable rhizosphere conditions. Differences in root exudation between different forest sites are believed to be driven by site-specific factors such as nutrient availability (Yin *et al.*, 2014). It was suggested that root exudation and

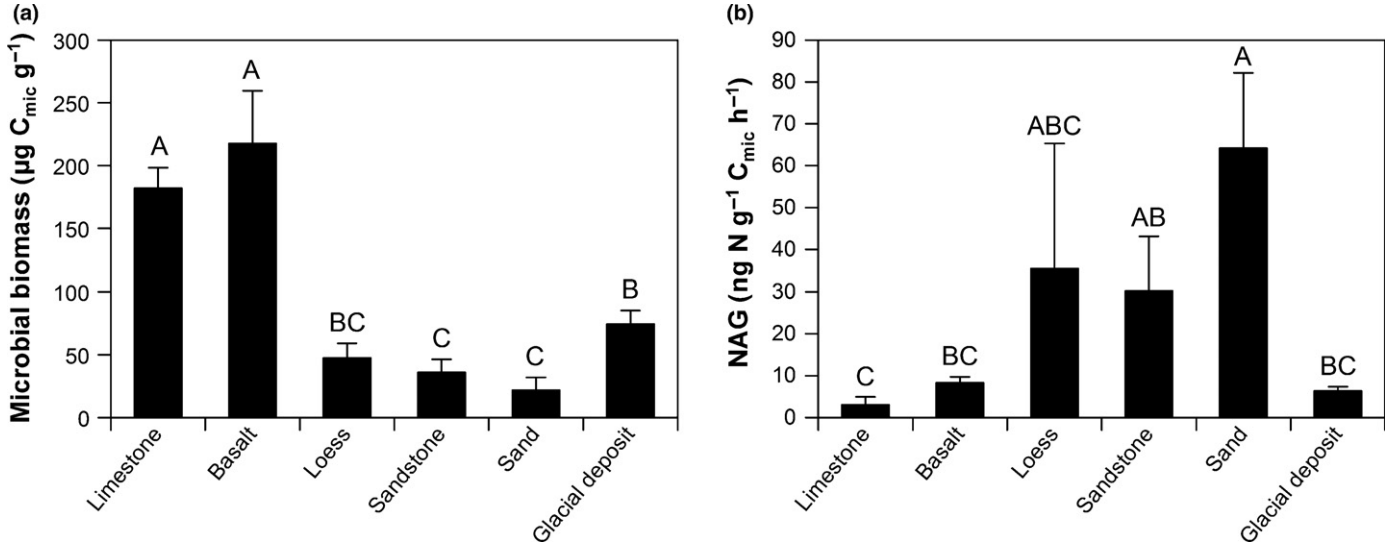

Fig. 4 Means + SE of (a) microbial biomass carbon (C) and (b) specific *N*-acetyl-*β*-glucosaminidase (NAG) activity per microbial biomass C in the topsoil of European beech forests on six different bedrock types ($n = 4$ pits per substrate). Study sites were sampled in June 2013 (glacial deposits) and May 2014 (all other substrates). Different upper-case letters indicate significant differences between the substrates.

Table 4 Multiple regression analyses with backward variable elimination on the effects of specific root length (SRL), mean annual temperature (MAT), pH ($CaCl_2$), C : N and *N*-acetyl-β-glucosaminidase (NAG) activity on root exudation rates and the estimated annual carbon flux from root exudation in the topsoil of European beech forests on six different bedrock types.

	Model					
	R^2	*P*		Predictor	*F*	*P*
Exudation rate	0.90	<0.001	–	pH	40.7	<0.001
			+	SRL	24.0	<0.001
			–	NAG	7.2	0.02
Annual flux	0.66	<0.001	+	SRL	15.7	0.001
			+	MAT	6.6	0.02

the associated priming effect respond to N deficiency (Dijkstra *et al.*, 2013; Canarini *et al.*, 2019). A low SON concentration increases the diffusion-driven (passive) exudation by a steeper concentration gradient between root cells and soil environment, which leads to increased exudation per unit root biomass at low N supply (Paterson & Sim, 2000). By contrast, N uptake in excess of immediate usage in growth processes, as it may occur in N-rich forest stands, reduces assimilate and C allocation to roots (Kuzyakov & Domanski, 2000; Nguyen, 2003) and consequently root exudation rates (Nakayama & Tateno, 2018). Hence, in accordance with the resource optimization theory, the C costs of root exudation for plants should be greater in nutrient-poor forest soils, where the majority of soil N is contained in organic N forms (*sensu* resource optimization theory; Ågren & Franklin, 2003). Enhanced C allocation to root exudation in nutrient-poor forests can stimulate the microbial decomposition of SOM through microbial priming effects and thus accelerate N-cycling and the release of soil N from less bioavailable sources (Phillips *et al.*, 2012; Meier *et al.*, 2017). The release of SON can occur without concomitant changes in microbial biomass, but just from the change in microbial production of extracellular enzymes. Elevated enzyme activity can increase the plant availability of organically bound N and increase the N uptake by trees in N-deficient forest stands (Finzi *et al.*, 2007). Experimental evidence has demonstrated increased root exudation under conditions of N-limited plant growth, which led to an increase in plant N when N could be mined from organic sources (Sun *et al.*, 2017). By contrast, the relationship between root exudation and plant N was negative when nitrate was the only N source, and enhanced exudation could not stimulate SON mining and enhance plant N uptake (Darwent *et al.*, 2003; see also Fransson & Johansson, 2010). Across the investigated nutrient availability gradient of the current study, which included soil N from both organic and inorganic sources, we found a positive relationship between the annual C flux from root exudation and soil C : N ratio, which points at an increase in root exudation when soil N is increasingly limiting for plant growth.

Surprisingly, we found no relationship between P availability and root exudation of beech trees. This is in contrast to previous studies, which showed that anion channel proteins significantly increase the passive efflux of carboxylates (e.g. citric acid, malic acid) in response to P deficiency, whose acidifying and chelating properties enhance the solubility of inorganic P (Neumann & Römheld, 2007; Lambers *et al.*, 2012; Zhang *et al.*, 2016). The exuded carboxylates can also serve as substrate for microorganisms to stimulate the microbial production of phytases and phosphatases – exoenzymes that catalyze the decomposition of organic P to phosphate. In addition, P-limited plants can also exude acid phosphatases (and sometimes phytases) directly (Miller *et al.*, 2001; Spohn & Kuzyakov, 2013). However, the bulk of evidence of greatly enhanced root exudation of carboxylates under P-limited soil conditions has been collected in non-mycorrhizal Proteaceae and arbuscular mycorrhizal crop plants (e.g. López-Bucio *et al.*, 2000; Lambers *et al.*, 2012; Zhang *et al.*, 2016), while ectomycorrhizal trees may rely more on their associated mycelia for exploring the soil and accessing immobile P resources (Cairney, 2011; Köhler *et al.*, 2018).

Low fungal-derived enzyme activities are associated with higher root exudation

Our study demonstrates that the activity of hydrolases, which depolymerize chitin and cellulose, is negatively related to root exudation rates in beech forests. The activity of NAG has often been associated with the biomass of mycorrhizal and saprotrophic fungi, which are the main producers of chitinases and polysaccharide hydrolases (Baldrian, 2008; Billings & Ziegler, 2008). We assume that beech trees release more root exudates on glacial deposits than on the other sites and that these sites can be characterized by lower fungal cleavage of C and N compounds in the bulk soil. The analysis of microbial biomarkers revealed a high biomass of fungi in the topsoil of the calcareous basalt site (fungal : bacterial ratio: 3.8; fungal biomass C: 172 $\mu g\,C\,g^{-1}$) and intermediate values in the topsoil of the loess and sand sites (fungal : bacterial ratio: 1.5–2.2; fungal biomass C: 15–28 $\mu g\,C\,g^{-1}$; based on the quantity of microbial biomarkers; Angst *et al.*, 2018). By contrast, fungal biomass was strongly reduced in the upper subsoil on the glacial deposits (fungal : bacterial ratio: 0.1; fungal biomass C: 0.18 $\mu g\,C\,g^{-1}$; based on $\delta^{13}C$ phospholipid fatty acid analyses; Preusser *et al.*, 2017). This decrease in fungal abundance from the base-rich calcareous to the highly acidic sandy site is probably related to the availability of SOC in the bulk soil, which is commonly the most limiting factor for microbial growth in soils (Ekblad & Nordgren, 2002; Demoling *et al.*, 2007; Preusser *et al.*, 2017). High availability of SOC may have promoted fungal growth at the basalt site, while both bacterial and fungal growth and activity in the bulk soil on the glacial deposits were probably limited primarily by low C supply from SOM, which restricted the synthesis of enzymes (cf. Heitkötter *et al.*, 2017). At these sites with C-deficient fungal activity, enhanced labile C supply from root exudation has the potential to trigger increases in decomposition rates and thus N availability (Phillips *et al.*, 2011; Chen *et al.*, 2014). This may indicate that elevated root exudation in the topsoil on the glacial sandy substrates represents an acclimation of the trees to low nutrient availability in general and the rapid nutrient impoverishment with

soil depth at these sites, where most of the nutrient uptake must take place in the thin AE horizon, which is enriched with organic material (Tückmantel *et al.*, 2017). Under these circumstances, a high root exudation activity may be vital for the stimulation of saprotrophic microbes and the continuous supply of nutrients to the trees.

Conclusion

Our study shows that the quantity of C released with root exudation is closely positively related to SRL and soil acidity and negatively to fungal abundance and activity. We demonstrate that in highly acidic soil more plant C is lost to the external ecosystem carbon cycle than in less acidic soils. Previous studies have suggested a decrease in mycorrhizal fungal colonization of roots in highly acidic soils (St Clair & Lynch, 2005; Carrino-Kyker *et al.*, 2016; Leberecht *et al.*, 2016). If this applies also to our study, reduced sink strength of mycorrhizal fungi for plant photosynthates in acidic soil may have increased root exudation rates – a hypothesis that has to be tested in future studies.

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Author contributions

ICM and CL planned and designed the overall research. BM, EK and CL planned and designed the grid sampling scheme. TT, JH and SP conducted fieldwork and laboratory analyses. ICM, TT, KM and TJW analyzed the data. ICM and CL wrote the manuscript. All authors annotated and approved the final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Temporal variation in root exudation rates.

Fig. S2 Relationship of SRL with root diameter and annual C flux from root exudation.

Fig. S3 Temporal variation in SRL.

Fig. S4 Relationship of root exudation with P_{resin} and AP activity.

Table S1 Estimated annual C flux from root exudation.

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