THE INFLUENCE OF TEMPERATURE, NUTRIENT AVAILABILITY AND SOIL DEPTH ON ROOT EXUDATION IN EUROPEAN BEECH FORESTS

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Timo Tückmantel

aus Haan

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Betreuungsausschuss

Prof. Dr. Christoph Leuschner,
Abteilung Pflanzenökologie und Ökosystemforschung, Georg-August-Universität Göttingen

Prof. Dr. Christian Ammer,
Abteilung Waldbau und Waldökologie der gemäßigten Zonen, Georg-August-Universität Göttingen

Prof. Dr. Ina Christin Meier,
Abteilung Funktionelle Waldökologie, Universität Hamburg

Mitglieder der Prüfungskommission

Referent: Prof. Dr. Christoph Leuschner,
Abteilung Pflanzenökologie und Ökosystemforschung, Georg-August-Universität Göttingen

Korreferent: Prof. Dr. Christian Ammer,
Abteilung Waldbau und Waldökologie der gemäßigten Zonen, Georg-August-Universität Göttingen

Weitere Mitglieder der Prüfungskommission

Prof. Dr. Ina Christin Meier,
Abteilung Funktionelle Waldökologie, Universität Hamburg

Prof. Dr. Erwin Bergmeier,
Abteilung Vegetationsanalyse & Phytodiversität, Georg-August-Universität Göttingen

Prof. Dr. Dirk Hölscher,
Abteilung Tropical Silviculture and Forest Ecology, Georg-August-Universität Göttingen

Prof. Dr. Mark Maraun
Abteilung J.F. Blumenbach Institute of Zoology and Anthropology, Georg-August-Universität Göttingen

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CHAPTER 1

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GENERAL INTRODUCTION

European beech (*Fagus sylvatica* L.)

The broad-leaved tree species with the highest abundance in Germany is european beech (*Fagus sylvatica* L.), which would be the dominant species on most sites due to its height, physiology and competitiveness (Ellenberg & Leuschner 2010). Beech is a late successional species and can age up to 300 years. It is a late fruiting species that reproduces mainly in the form of irregular masting events (Ellenberg & Leuschner 2010). The main natural distribution of beech is in central and western Europe with atlantic to subcontinental climate. In Germany, it is absent in the high altitudes of the low mountain ranges and the Alps, on azonal, as well as on extremely wet and dry sites. In southern and southeastern Europe, its distribution is restricted to the montane stage. Most of today's populations in Germany are located at an altitude of 200 to 800 m a.s.l., in the Schwarzwald their occurrence ranges from 150 to 1400 m a.s.l. (Dittmar et al., 2001). Successful growth of beech depends on sufficiently long growing seasons of at least 140 days (Ellenberg and Leuschner 2010, Fotelli et al 2003, Peuke et al 2006). Beech trees show high tolerance to different soil types. They grow on acidic to neutral up to slightly alkaline soils, as well as on nutrient rich to poor soils. This is because under certain conditions, such as sufficiently moist soil and a minimum of organic matter, beech trees can form an almost closed nutrient cycle with the help of their fine root network (Ellenberg and Leuschner 2010). Compared to other forest trees, beech has the distinct advantage that its seedlings are very shade tolerant, while mature trees form closed canopies that capture the majority of usable light. European beech is a tree species associated with ectomycorrhiza (ECM) (Liese et al., 2017). ECM-associated trees are characterized by slow decomposing leaf litter and a large amount of organically bound nutrients in the soil, which can be mined by plants from the degradation of soil organic material by extracellular enzymes that are produced by mycorrhizae (Cornelissen et al., 2001; Phillips et al., 2013; Yin et al., 2014).

Beech has high economic importance for the forestry and timber industry and is frequently planted again due to its good properties (Dittmar et al., 2001). In the course of climate change, however, the distribution of beech could change. Changing weather phenomena and the alternating phases of moisture and drought that accompany them imply a risk for existing beech stands that is rooted in the drought sensitivity of *Fagus sylvatica* (Ellenberg and Leuschner 2010, Scharnweber et al 2011, Zapater et al 2012). It is assumed that by the end of the century, european beech will not be able to persist in all places where it dominates today (Rennenberg et al. 2004). This will be due, among other things, to reduced nutrient availability and

mobility; thus, reduced water availability causes reduced nitrogen (N) uptake, which inhibits plant growth (Hacke & Sauter 1995; Fotelli et al., 2001, 2002).

Priming effects

The rhizosphere priming effect (RPE) describes a close-proximity short-term change in soil organic matter (SOM) decomposition rates as a response to labile carbon (C) inputs, deriving from living roots (Kuzyakov et al., 2000). In this context, the "nutrient mining" theory is discussed, which is based on the assumption that microbial growth is promoted by the input of exogenous, labile C as an energy source. The consequence is a limitation of other nutrients such as nitrogen (N), which triggers microbial nutrient mining of SOM and thus priming effects (Blagodatskaya & Kuzyakov, 2008; Wang et al., 2015). Rhizosphere denotes the closest soil material surrounding the roots, where a variety of complex biological and ecological processes occur, which get affected by activities of the root (Eldhuset et al., 2007). Within the rhizosphere, interactions between roots and pathogenic and beneficial soil microfauna, invertebrates and root systems of competitors take place on a highly dynamic scale (Hirsch et al., 2003; Bais et al., 2006). Conditioned by a permanent outflow of labile carbon compounds, the rhizosphere is characterized by a surplus that can cause positive, negative, or neutral RPE. RPE with positive effects are considered as the result of co-metabolisms between soil organic carbon (SOC) and rhizodeposited C, activation of SOC-accessing and decomposing microbes, or N mining from SOC under limitation (Murphy et al., 2015; Wang et al., 2016). Negative Effects, in contrast, seem to be the result of preference of rhizodeposits over SOC by microbes and N limited SOC decomposition, resulting from N competition between plants and microorganisms (Schimel et al., 1989). Microbial activity as a reason for positive RPE is supported by observed increases in microbial biomass associating positive RPE´s (Kuzyakov, 2010). Significant sites for priming effects are characterized by exceptionally high microbial quality and quantity, which can be, for example, found within the detritusphere (characterized by a strong gradient of C-concentration from litter (high) into the adjacent soil (lower)) (Kuzyakov, 2010). Communities of soil microbes are generally often limited in energy rich Carbon compounds (Demoling et al., 2007; H. Wang et al., 2015) so that microorganisms therefore get attracted by substances deposited by plant roots. Bacteria are the first to retain and metabolize easily available organic substances, as demonstrated by ^{13}C incorporation (Paterson et al., 2007; Moore-Kucera & Dick, 2008), which accelerate the turnover of bacterial biomass. Fungi can degrade organic material that is poorly available to the majority of

bacteria, including bacterial necromass, which make fungi secondary profiteers of easily degradable substances. Furthermore, fungi have the advantage over bacteria that they can explore other areas of the soil through the growth of their hyphae and thus significantly increase the radius of action (Otten et al., 2001). It must be considered, that within both groups, bacteria and fungi, various species are substrate-specialists and decompose material differently, sometimes even capable of switching preferences depending on the substrate present (Kuzyakov, 2010).

Rhizodeposition and Exudates

Rhizodeposition has a fundamental role for C and nutrient cycling. Deposited substances get incorporated into microorganisms, SOM or decomposed to CO_2 quickly. There are relationships between plant species identity and the quantity of allocated C, but the further fate within the soil is only conditionally dependent on the species, rather by given rhizosphere conditions (Pausch & Kuzyakov, 2018). Most of the biochemical and physical differences between soil material and rhizosphere are caused by the release of different types of rhizodeposits. Rhizodeposits consist of a wide range of compounds, released from roots into the soil, serving a variety of ecological purposes (Bais et al., 2006). Components of rhizodeposits can be classified into water-insoluble materials (e.g., mucilage, (sloughed) cells and dying fine roots) and water-soluble exudates (e.g. organic but also inorganic compounds) (Smith, 1976; Merbach et al., 1999; Wichern et al., 2008; Preece et al., 2018). Larger components like sloughed cells, root hairs, mucilage or decomposing plant material provide a predominant role in processes mediated by microbes in soil, supplying heterotrophic organisms (Pausch & Kuzyakov, 2018; X. Wang et al., 2016). Exudates are released from the tips of growing roots and have profound impact on the properties of the rhizosphere. Their type can be broadly divided into two processes: root excretion or basal exudation, which include output of substances with unknown function in dependency of gradients between root and soil, and, furthermore, root secretion, which means the output of known substances, which is usually mediated via membrane-bound channels (Bais et al., 2004, 2006; Jones et al. 2004). Plants exude appreciable contents of their assimilated C from their roots, magnitudes of excreted photosynthates vary with soil conditions, age and physiological state of the plant as well as nutrient availability (Bais et al., 2006). Observed magnitudes were 2-11 % (Jones et al., 2004; Jones et al., 2009; Preece et al., 2018) and 20-40 % (Canarini et al., 2019; Prescott et al., 2020). Study results suggest, that increased fluxes of photosynthates into the root under limiting environmental

conditions can increment exudation rates, for example, Juszczuk et al. (2004) found close relations between soluble sugar inflow into fine roots and exudate amount. Quality and quantity of released compounds vary considerably between different tree taxa (Smith, 1976) and further depend on the type of mycorrhial association, environmental and soil conditions (Qiao et al., 2014). Exposed to drought, ECM trees were found to exude almost twofold higher, accompanied by a strong increase in photosynthetic cost (Liese et al., 2017).

A large number of different compounds can be found in plant exudates, which can basically be divided into two groups. First, low molecular weight compounds (e.g., amino acids, org. acids, sugars, phenolics and other secondary metabolites) which represent the majority of diverseness in substances and second, high-molecular weight exudates, such as polysaccharides and proteins (e.g., enzymes) which represent the quantitative majority, although being less diverse (Bais et al., 2006). Soil Bacteria preferably trap and metabolize easily degradable, energy-rich organic compounds like monosaccharides, which increases turnover and in consequence the degradation of SOM. Some rhizobacteria improve plant growth by providing benefits like nutrients. Exuded amino acids and carbohydrates were observed to attract bacteria on root surfaces via chemotaxis (Somers et al., 2004).

Organic acids (this denotes for both the undissociated acid and the dissociated anion) released are often of low molecular weight and monocarboxylates represent the majority. For instance, butyric, formic, lactic, malonic, oxalic, phtalic and shikimic acids were documented for tree root exudates (Sandnes et al., 2005). The release of organic acids has a role in nutrient acquisition, like phosphorus (P) or iron (FE), and detoxification of harmful elements. P is often bound in ferric or aluminium phosphates, especially in soils characterized by higher pH-values. Organic acids can release P via complex alteration, releasing plant-available P into the soil. (Jones & Darrah, 1994; Jones, 1998; Bais et al., 2006; Eldhuset et al., 2007). The amount of exuded organic acids is depending on a variety of different factors, for instance tree species, developmental stage, root density, mycorrhizal status and growing conditions (Sandnes et al., 2005). For example, Eldhuset et al. (2007) found high amounts of organic acid concentrations in exudates, thereby especially oxalate, of *Picea abies*, depending on mycorrhization in high aluminium (Al) growing conditions and were interpreted to be relevant for Al resistance via elevated oxalate contents in the rhizosphere.

Plant roots exude extracellular enzymes directly, for instance, to increase SOM decomposition, (Kuzyakov, 2010), or release substances, which promote fungal growth, which in turn release enzymes affecting SOM.

Phytosiderophores, as an example for exuded secondary metabolites, are capable of chelating

metallic micronutrients, directly or via complex alteration, which increase the mobility and useability of lesser available resources. For instance, graminoid phytosiderophores raise as a chelator iron (Fe) availability (Romheld & Marschner, 1986; Bais et al., 2006). Some secondary metabolites, released from the root, may have allelopathic functions, they can have a phytotoxic effect, which is considered as a mechanism to gain an advantage over competitors. The following compounds are known in this context: 7,8-benzoflavone (*Acroptilon repens*), juglone (*Juglans nigra*), 8-hydroxyquinoline (*Centaurea diffusa*) or sorgoleone (*Sorghum* spp.) (Bais et al., 2006).

The composition of root exudate components varies under different nutritional conditions. It has been reported that plants under N limitation exude amino acids slower (Bowen, 1969; Haase et al., 2007), P limitation can lead to an increase in carbohydrate (maize) (Carvalhais et al., 2011) or amino acid (cotton) (Yan et al., 2007) release.

Root Exudation and ecological conditions

The effects and benefits exerted by root exudates in soils have been studied extensively in the past, in terms of direct and indirect effects, chemical composition, and how they condition both promoting and inhibiting effects in the rhizosphere. To a much lesser extent, the dynamics of interactions between plant ecological conditions with the pattern of root-derived carbon release has been studied.

Soils are often characterized by high heterogeneity in the distribution of nutrients and often provide an inadequate supply of N, in relation to the needs of the plant, so that the demand for nutrients must be fulfilled from the decomposition of SOM (Murphy et al., 2015). This nutrient release, especially of N, can be enhanced by exuded carbon and consequently increased microbial decomposition rates. The spatial and temporal distribution of SOM in the soil is highly variable, it consists of a heterogeneous mixture of essential nutrients (Lal, 2009; Murphy et al., 2015; Schmidt et al., 2011), which can be exploited to a variable extend. The quantity and quality of exuded compounds must therefore be adapted to the availability of SOM and its composition, regarding optimized efficiency. However, the extent to which nutrient availability, as well as the presence, accessibility, and distribution within the soil condition spatial flexibility in exudate excretion has hardly been studied. Root exudation consists of a diffusion driven basal component, compounds, which have a direct effect, such as organic acids, and to a considerable extent compounds whose effects are mainly found in the priming

of microorganisms. (see chapter 2.1.). The need, by means of magnitude of exudation, to prime microorganisms furtherefore depends on whether nutrients are in a biodegradable form of SOM. Since these are distributed differently depending on the soil type or depth, or can be replaced by more bioavailable nutrient forms, the abundance and spatial distribution of SOM should be reflected in the exudation.
There is also evidence that climatic conditions affect the amount of carbon exuded. For example, connections were found between mild water shortages and droughts, which mostly led to an increase in exudation rates (Preece & Peñuelas, 2016; Liese et al., 2017; Preece et al., 2018). Temperature effects could also be found, but study results supporting these connections mainly derived from common garden experiments with seedlings or saplings (Liu et al., 2021; Xiong et al., 2020; Yin et al., 2014). In most cases increases in ambient temperatures were found to be connected to increased exudation rates, but they are only comparable to a limited extend with *in situ* conditions of mature trees.

With the aim of investigating different influences on root exudation pattern, three transect studies with gradual approaches were initiated. In a first project, information was to be collected on how the spatial distribution of nutrients and minable material in the subsoil affect root derived carbon outflow and to what extent root morphology and abundance influence this. In a second project, soil types, which originated from different geological source materials, characterized by different soil chemistry and nutrient availability, were analyzed for their influence on the quantity of exuded carbon and related to fine root morphology. In a third project, climatic influences on the quantity of root-derived carbon fluxes were to be investigated. The focus laid on the temperature regime and whether long-term adaptation to climate regimes at different locations or short-term changes in local temperature were important.

Methodical approach

To investigate the complex ecological relationships that exist around fine root-derived carbon fluxes, edaphic and climatic factors have been addressed in three separate studies.
The examination of gradual changes in key exogenous factors under otherwise similar conditions was selected as the methodological basis for receiving information on the relationships between exudation and closely linked fine root morphology on the one hand, and edaphic factors varying according to soil depth (A) and nutrient availability (B), as well as climatic factors (C), especially temperature, on the other hand.

A) A field study with a soil depth gradient near Nienburg (Weser), associated to a cooperative project (SUBSOM) dealing with organic matter storage and turnover in the subsoil.

B) A second field study was carried out, using different soils resulting from the weathering of different bedrock materials and forming a gradient in nutrient availability. This project was also associated to SUBSOM and took place on different sites distributed around Göttingen in addition to the site near Nienburg.

C) An elevation transect was established for the third field study in northwestern Hesse between Willingen and the vicinity of Korbach, which was characterized by a distinct temperature and precipitation gradient.

Mature beech forests comparable in age, stand structure and soil development since the Holocene were studied in these projects. Morphological root traits as well as fine root biomasses in the soil and exudation rates were quantified. Furthermore, edaphic parameters characterizing soil chemistry and physics as well as nutrient contents were determined. Supporting cooperation within the framework of the SUBSOM project provided additional data such as soil microbial biomass and (exo-) enzyme activities, which were highly useful to interpret results (chapters 2 and 3). Climate data were measured within the stands of the elevational transect and, in addition, obtained from the german weather service (DWD – deutscher Wetterdienst).

Soil depth gradient

Study area

The research was conducted in a mature beech forest established in 1916 (Nienburg 2010) in the lowlands of NW Germany (52°14'N, 9°20'E; 100 m a.s.l.) in the `Grinderwald´, a 1000 ha forest at 106 m a.s.l.

The site has a cool-temperate climate and mean annual precipitation of 713 mm (MAP) and mean annual temperature of 9.4°C (MAT) (DWD, period of 1947-2015). The study years of 2014 and 2015 had warmer temperatures with 2014 surpassing long term mean by 1.7°C and precipitation of 422 mm in 2014 and 371 mm in 2015.

Soils were developed from Pleistocene fluvioglacial sandy deposits from the penultimate (Saalian) ice age as parent material. Sandy deposits were medium-to coarse-grained with low silt and clay content and were characterized by a comparably low water storage capacity. The soil type was an acidic sandy dystric cambisol (pH 3.4e4.5) (IUSS Working Group WRB, 2014) with a small AE horizon (2 cm; represents the topsoil). Soil below the A and E horizons was defined as subsoil.
Mormoder was the dominating humus form (classification according to Green et al., 1993). Soil manipulations like e.g. liming were absent.

Field sampling

In 2014 and 2015, four sampling campaigns were conducted to obtain root exudates. For this purpose, three pits with a depth of approximately two meters were excavated in each year with a maximal distance of 3 m from the nearest beech. Twice in both years, three root strands were carefully exposed in the pit walls, and categorized in three depth classes (topsoil, 20-50 cm, and 60-130 cm), which had to be established as a consequence of irregular root distribution in the subsoil. The terminal regions of these root strands were carefully cleaned and utilized to collect exudates. Subsequently, the sampled roots were severed and transferred to the laboratory for root morphology analysis.
Soil sampling was conducted horizontally within the pit walls at depths of 5, 45, and 110 cm in June 2015.

Figure 1 Exemplified collection of root exudates the layer of 20-50 cm depth. Sampling cuvettes (briefly uncovered) with embedded roots and associated sterile collection vessels attached to terminal zones of root strands.

Measured parameters

The focus of this research project laid on the investigation of factors, that shift along increasing soil depth like organic matter composition, soil chemistry, physics and root exudation pattern and root morphology and to search for possible relationships. The following parameters were measured during this study:

- Quantity of released C by exudation and annual C fluxes
- Root architecture and morphology: fine root diameter, root tissue density, specific root length and specific root area
- Soil characteristica: microbial biomass C, extractable organic C (EOC), and extractable N (ETN), Phosphorus (P) availability
- Soil fractionation and chemical composition: Soil organic carbon (SOC), mineral associated organic carbon (mineral OC), particulate organic carbon (POM), according to (Angst et al., 2016)

Bedrock gradient

Study area

The study areas for this project were located in the vicinity of Göttingen in Lower Saxony with the addition of the area near Nienburg, which was used for the depth gradient. Thereby, the investigations took place in seven soils, which had emerged from different parent materials and were set at comparable heights (pleistocene sands (52°14'N, 9°20'E; 100 m a.s.l.), tertiary sands (52°26'N, 9°41'E; 270 m a.s.l.), quartenary loess (51°34'N, 10°14'E; 200 m a.s.l.), triassic sandstone (51°34'N, 10°03'E; 295 m a.s.l.), tertiary basalt (51°28'N, 9°45'E; 470 m a.s.l.) and triassic limestone (51°32'N, 10°02'E; 410 m a.s.l.)). Soil development proceeded since the Holocene.

Beech stands were of matured age between 95 and 166 years at the time of sampling, dominated by *Fagus sylvatica*, with only minor admixture of other species. However, within stands, plots free of other tree species were selected for comparability. The mean stem diameter of trees ranged from 33.1 to 50.2 cm and stem density ranged from 111 to 300 ha^{-1}, and cumulative basal area ranged from 22.8 to 43.2 m2 ha^{-1}.

All sites were selected in the cool-temperate climate zone under comparable conditions with mean annual precipitation between 709 and 902 mm (MAP) and mean annual temperature between 7.1 and 8.7 °C (MAT). Additional influences due to different climatic conditions (i.e. exposition, inclination) were to be minimized.

Three of the sites were characterized by rather shallow soil profiles (< 80 cm) (sandstone, basalt, limestone), while the other three (glacial sediments, tertiary sands, loess) had soil profiles greater than 2 m deep. Table 1.1 provides an overview of specific site information, e.g. soil type, humus form and nutrient availability.

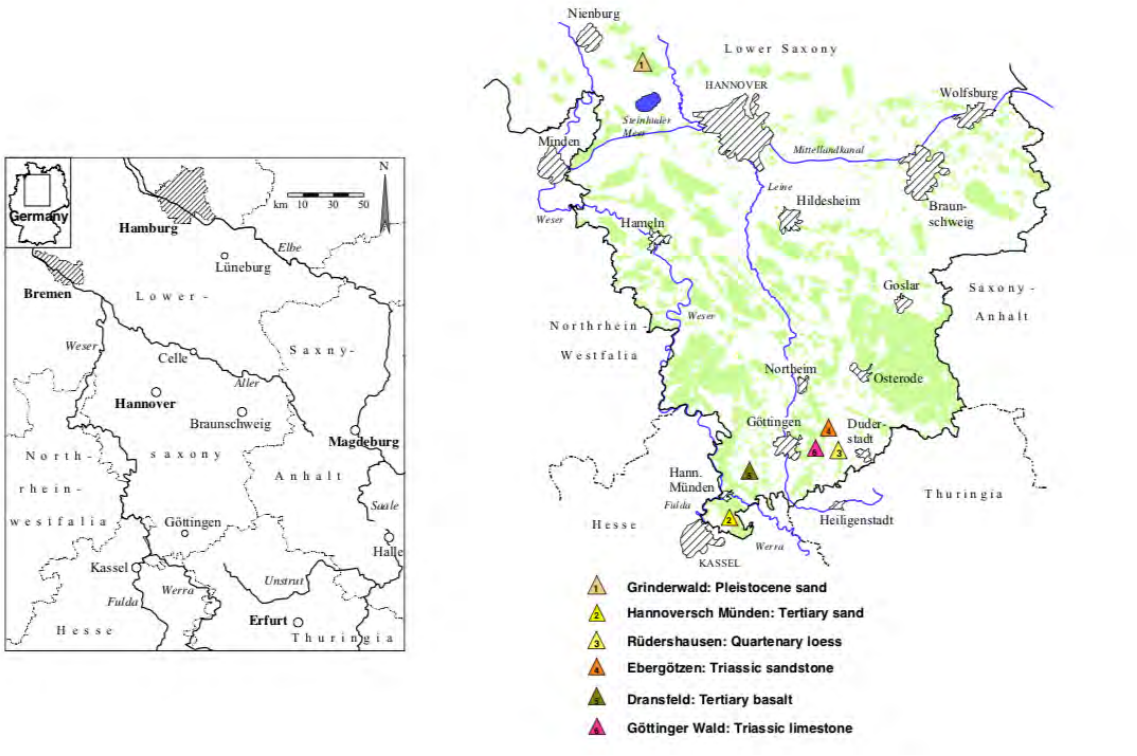

Figure 2 Distribution of experimental plots of soil depth and bedrock type gradients in the vicinity of Göttingen and near Nienburg.

Source: Dietrich Hertel, unpublished

Field sampling

The collection of root exudate samples was conducted in three campaigns in 2014 and 2015. With a maximum distance of three meters from the trunk of the selected tree, three samples and one blank were obtained each time, with a total of three trees per site and measurement campaign.

To limit the possibility of accidentally sampling other tree individuals, small-scale sites were investigated that provided the widest possible distances from neighboring trees but were still located under closed canopy cover.

Small depressions were created where the overlying leaf litter and, if necessary, parts of the organic soil layer were removed until individual root strands could be exposed undamaged. Soil sampling for nutrient analyses was carried out in May 2014.

Plot no.	1	2	3	4	5	6
Site	Grinderwald (Gr)	Hann.Muenden (HM)	Ruedershausen (Ru)	Ebergoetzen (EG)	Dransfeld (Dr)	Goettinger Wald (GW)
Bedrock	Pleistocene glacio-fluviatile deposits (Saale)	Tertiary sand	Quaternary loess	Triassic sandstone	Tertiary basalt	Triassic limestone
Soil type [1]	Dystric Cambisol	Cambisol	Cambisol	Cambisol	Cambisol	Chromic Cambisol
Organic layer	Leptomoder	Hemimor	Leptomoder	Leptomoder	Mullmoder	Vermimull
Thickness of organic layer (mm)	35	44	20	19	37	18
Maximum profile depth (m)	≥ 2	≥ 2	≥ 2	60-80	60-80	60-80
Upper subsoil (m)	20 - 110	20 - 110	20 - 110	20 - 50	20 - 50	20 - 50
Lower subsoil (m)	110 - 200	110 - 200	110 - 200	50 - 80	50 - 80	50 - 80
SOC (%)						
Topsoil (0-20 cm)	1.15	1.60	0.99	1.40	3.60	2.50
Upper subsoil	0.20	0.25	0.46	0.33	2.10	1.60
Lower subsoil	0.06	0.05	0.33	0.13	1.30	1.40
C/N						
Topsoil (0-20 cm)	26.3	20.8	12.8	17.4	14.1	13.6
Upper subsoil	14.3	12.3	8.2	10.7	13.2	11.4
Lower subsoil	13.1	8.9	5.7	5.4	15.0	11.6
pH ($CaCl_2$)						
Topsoil (0-20 cm)	3.5	3.7	3.6	4.0	3.7	4.3
Upper subsoil	4.1	4.1	3.7	3.9	4.1	5.5
Lower subsoil	4.0	3.9	4.0	3.8	4.8	6.6
Texture [1]						
Topsoil (0-20 cm)	Sandy loam	Sandy loam	Silt	Loam	Silt loam	Silt loam
Upper subsoil	Loamy sand	Sandy loam	Silt	Silt loam	Silt loam	Silt
Lower subsoil	Loamy sand	Sandy loam	Silt	Silt loam	Silt	Silt
Bulk density (g cm^{-3})						
Topsoil (0-20 cm)	1.2	1.2	1.1	1.1	n.a	1.2
Upper subsoil	1.5	1.4	1.4	1.5	n.a	1.4
Lower subsoil	1.5	1.4	1.5	1.2	n.a	1.3
Cation exchange capacity (µmolc g^{-1})						
Topsoil (0-20 cm)		32.6	52.8	50.9	100.1	117.8
Upper subsoil		10.4	78.6	52.2	83.9	303.5
Lower subsoil		12.2	98.7	84.7	175.9	204.3
Base saturation (%)						
Topsoil (0-20 cm)		4.8	16.3	26.8	14.2	57.4
Upper subsoil		9.3	39.4	24.5	51.0	99.8
Lower subsoil		10.7	99.5	22.7	97.5	96.5

Table 1 Overview of site characteristics, soil chemistry and nutrient contents along the bedrock gradient

Source: Dietrich Hertel, unpublished

Measured parameters

The purpose of this study was to gain insight into the extent to which the properties of different soil types, varying substantially in soil chemistry and nutrient availability, are related to the amount of root-borne exuded carbon and the formation of fine root morphology, and whether and to what extent relationships between these parameters are detectable. Furthermore, thanks to the cooperation within the "SUBSOM" project, additional information such as soil enzyme activity and microbial biomass could be accessed and used for the interpretation. The following factors are presented representatively:

- Quantity of released C by exudation and annual C fluxes
- Rooting parameters: fine root biomass in soil, fine root diameter and specific root length
- Soil characteristica: microbial biomass C, exoenzyme activity, soil organic C (SOC), and soil organic N (SON), C : N ratio and P availability

Elevation Transect

Study area

Seven research sites were selected and established with the goal of creating a study transect that would highlight primarily gradual differences of a climatic nature under conditions that were as comparable as possible, such as soil material, terrain relief and stand structure, as well as age. These were located in an east-west direction in northern Hesse, roughly outlined from the vicinity of the town of Korbach to northwest of Willingen with a maximal estimated distance of 30 km. Matured beech forests on acidic soils were distributed over altitudes from 300 to 800 m a.s.l. and ranged from the colline to the montane zone. The study region had a cold-temperate humid climate with MAT decreasing from 8.4 to 6.0°C and a mean annual precipitation (MAP) increasing from 600 to 1200 mm yr^{-1}. Predominant tree stands had an average age between 100 and 180 years and a mean diameter at breast height (dbh) of 32 to 45 cm. Stem density was more variable (150 - 580 ha^{-1}) which was compensated by selecting similar small-scale plots in areas of comparable density. There was no interference from other tree species in the examined areas. Soil formation occurred from two different bedrock types, Triassic sandstone at the two lowest sites and Paleozoic clay shale on the other five. Soil chemistry and nutrient availability analyses confirmed comparable conditions, except soil C and N contents, despite different parent bedrock material.

Field sampling

Small-scale survey plots of 30 x 30 m were selected within the selected sites under closed canopy. Three sampling campaigns were carried out in July 2014, August and September 2015, for which three trees per plot were used for exudate collection. At a distance of 3 m from the nearest beech tree, three small depressions were created (total of 9 per site), but only the uppermost layers were removed until the end of a root strand could carefully be uncovered without damage.

In addition to the soil sampling that took place preliminary the surveys, further soil samples were taken in 2015, attached to the exudate sampling, in order to investigate soil chemistry and nutrient availability. For this purpose, at each site five soil cores were taken to a depth of 15 cm.

To quantify soil rooting patterns, a total of twelve soil cores were extracted in November 2018 at various randomly distributed locations within the 30 x 30m plots and separated into

organic layer and upper mineral topsoil (0-10 cm). Fine roots collected from the sampled soil material were set in relation to the sampled soil to calculate root distribution.

For the determination of soil water content, five soil cores per site were collected monthly from March to December 2015, separated into organic layer and mineral topsoil, and determined gravimetrically. Collection in August and September 2015 was conducted synchronously with collection of exudates.

Small-scale, site-specific temperature data were measured using iButton sensors (Maxim, Dallas, USA) in both topsoil (3cm depth) and air (1.5 m height). Grid data of different temporal resolution from the German Weather Service (DWD) were used to calculate site-specific, longer-termed temperature and precipitation averages.

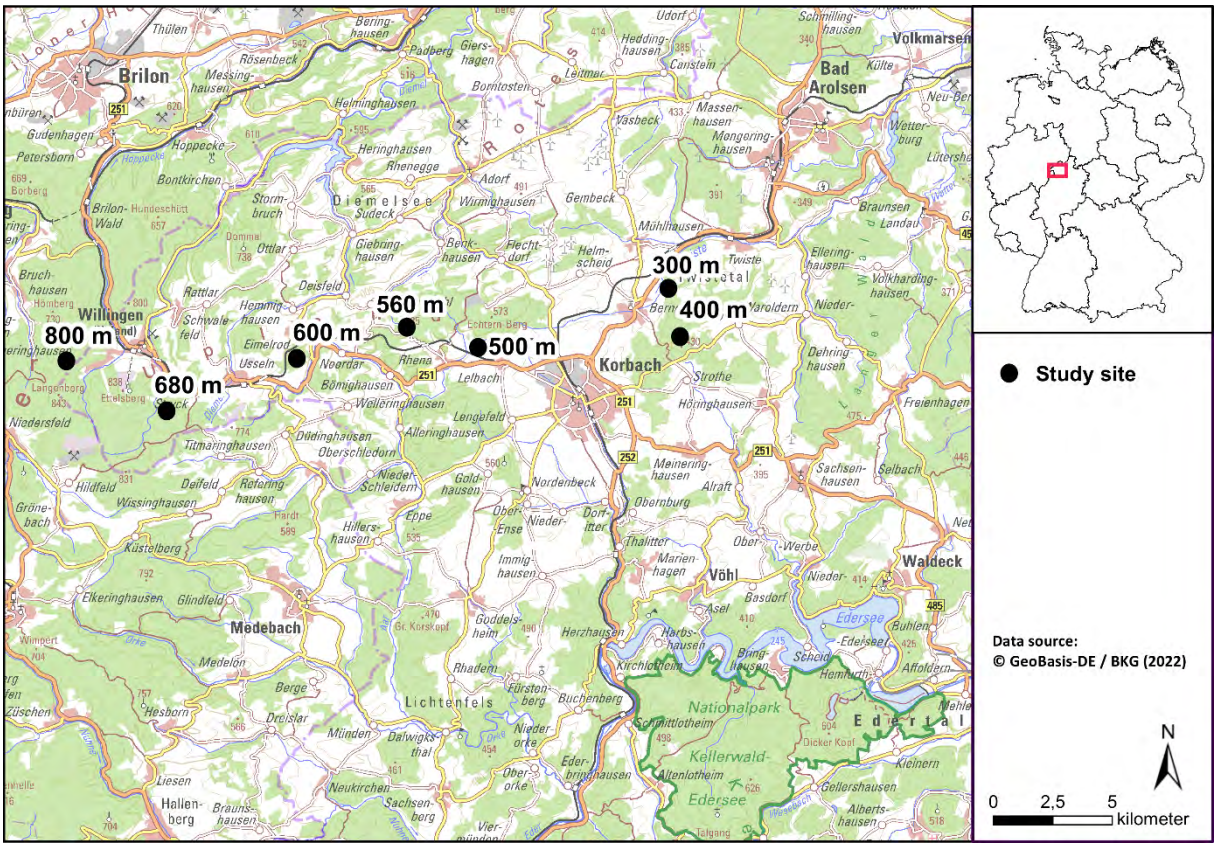

Figure 3 Location of the sites of the elevational gradient in northern Hesse; *Source: Julia Köhler, unpublished*

Measured parameter

In this study, influences of climatic nature on root-borne carbon fluxes were to be investigated. In addition to the determination of soil moisture and the evaluation of precipitation and long-term temperature data, priority was given to small-scale air and soil temperature data collected in the stands in order to be able to observe relationships locally. Investigated parameters are among others:

- Quantity of released C by exudation and annual C fluxes
- Rooting parameters: fine root biomass in soil, fine root diameter and specific root length
- Soil characteristica: pH, nutrient, C and soil water contents
- Long term mean annual temperature and precipitation, mean summer temperatures, prevalent air and soil temperatures

CHAPTER 2

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ROOT EXUDATION PATTERNS IN A BEECH FOREST: DEPENDENCE ON SOIL DEPTH, ROOT MORPHOLOGY, AND ENVIRONMENT

Timo Tückmantel · Christoph Leuschner · Sebastian Preusser · Ellen Kandeler · Gerrit Angst · Carsten W. Mueller · Ina Christin Meier

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Abstract

Forest subsoils may represent an important C sink in a warming world, but rhizodeposition as the key biogeochemical process determining the C sink strength of mature forests has not yet been quantified in subsoils. According to studies conducted in topsoil or laboratory experiments, soil C inputs by root exudation are increasing with increasing temperature and decreasing nutrient availability. We examined whether these relationships apply to forest subsoil by analyzing the response of root exudation to increasing soil depth up to 130 cm in a mature European beech (*Fagus sylvatica* L.) forest. In two subsequent growing seasons differing in temperature and precipitation, we investigated in situ root exudation with a cuvette-based method and analyzed root morphology, microbial biomass, and soil nutrient availability. We proved that root exudation greatly decreases with soil depth as a consequence of a significant decrease in root-mass specific exudation activity to nearly a fifth of topsoil activity. The decrease in specific metabolic activity from 312 mg C g^{-1} yr^{-1} in the topsoil to 80 mg C g^{-1} yr^{-1} at 130 cm depth was amplified by an exponential decrease in root biomass per soil volume, leading to a relative decrease in root exudation per volume in the deep subsoil to 2% of topsoil root exudation (1 g C 10 cm^{-1} m^{-2} yr^{-1} at 130 cm depth). Specific root area decreased and mean fine root diameter and root tissue density increased with soil depth, indicating a shift in primary root functionality from fibrous roots in the topsoil to pioneer roots in the subsoil. The decrease in root exudation was accompanied by de- creases in soil microbial biomass, extractable organic C (EOC), and N and P availability and increases in the aromatic C portion in SOM, but it did not relate to seasonal differences in climatic conditions. More specifically, it responded positively to an increase in EOC and ETN in the topsoil, but remained at its minimum rate in the SOC-poor subsoil, probably due to a lower organic N and higher mineral N content. The vertical pattern of beech root exudation is in accordance with a strategy to maximize whole-tree carbon-use efficiency, as it reduces C loss by exudation in soil spots where positive priming effects are unlikely, but enhances C exudation where microbes can mine less bioavailable SOM. The exudation patterns further suggest that increased C allocation to root systems as a likely tree response to elevated atmospheric [CO_2] may not lead to enhanced soil C input by root exudation to subsoils poor in SOM.

Keywords:
fagus sylvatica, nitrogen, pioneer roots, rhizodeposition, soc, subsoil

Introduction

Trees can increase the potential of soils as key global C sink under global warming. Forest soils store up to 70% of all soil organic C (SOC; Jobbagy and Jackson, 2000) and a considerable part of it in subsoils (in excess of 50%; Jobbagy and Jackson, 2000; Salome et al., 2010; Rumpel and Kögel-Knabner, 2011). Yet even though the sta- bility and control of subsoil organic C in forests has received increasing attention in recent years (e.g., Fontaine et al., 2007), quantitative information on plant C inputs to forest subsoils is still scarce. While it is well established that roots exert significant control on the rate at which C cycles between plants, soil, and the atmosphere (Norby and Jackson, 2000; Lal, 2004), how these relationships change from topsoil to subsoil is largely unknown. In particular the process of rhizodeposition which determines the C amount and C sink strength of forest subsoils has remained unstudied in mature forest stands due to methodical constraints.

In the topsoil, roots supply microbes with easily degradable C- rich substrates that drive microbial decomposition processes (Lynch and Whipps, 1990; Kong and Six, 2010; Phillips et al., 2012; Meier et al., 2015, 2017). As a consequence, biogeochemical cycles are much faster in the rhizosphere than in the surrounding bulk soil (Herman et al., 2006; Finzi et al., 2015). Greater soil C inputs by roots, e.g. as a consequence of elevated CO2, do not necessarily lead to increased C sequestration (Langley et al., 2009; Van Kessel et al., 2006; Marhan et al., 2010) since the exudation of labile, plant- derived C and root turnover can stimulate microbes to decompose less bioavailable SOC (Hoosbeek et al., 2004; Joslin et al., 2006; Phillips et al., 2011, 2012) via a priming effect (Kuzyakov et al., 2000). Priming effects can be positive (increase in SOC decomposition) or negative (slow-down of SOC decomposition) and can vary in magnitude (Cheng et al., 2003; Hamer and Marschner, 2005; Blagodatskaya et al., 2007; De Graaff et al., 2010, 2014). Both the direction and magnitude probably depend on the quantity and quality of the deposited root substrate, the microbial community composition and activity, and the quality and availability of soil C (Fierer et al., 2003; Fontaine et al., 2003; Hamer and Marschner, 2004;DeGraaff et al.,2010;Salome et al.,2010),allofwhich change with increasing soil depth.

Soil organic matter in deep soil is highly processed and several studies suggest that it is enriched in microbial-derived C com- pounds and depleted in energy-rich plant material in comparison to topsoil SOM (Rumpel and Kögel-Knabner, 2011). The main pathways by which new organic C inputs to subsoils occur are from leaf and root litter and root exudation (Rumpel and Kögel-Knabner, 2011; Angst et al., 2016a). As a consequence, rooting patterns control the vertical

distribution of organic matter and nutrients in the soil (Iversen, 2010) and their occurrence in subsoils is highly heterogeneous and mainly confined to hotspots. Spatial separation of SOM, microorganisms, and their extracellular enzyme activities possibly related to the heterogeneity of the root C input is discussed as one of the most important factors leading to the protection of SOM in subsoils (Von Lützow et al., 2006; Salome et al., 2010; Rumpel and Kögel-Knabner, 2011; Preusser et al., 2017).

Root morphology has a strong control on the C flux from roots to soil: increased root branching can increase root exudation rates (Groleau-Renaud et al., 1998) and promote fine root turnover and decomposition in the topsoil (Wells and Eissenstat, 2001; Guo et al., 2008; Fan and Guo, 2010; De Graaff et al., 2013), but it is unknown if the same relationships also exist in the subsoil with vastly different environmental conditions. While the complex architecture of root systems traditionally has been categorized according to root diameter into fine and coarse roots, this classification may not reflect the functionality of roots. More recently, fine roots were classified according to a stream-based ordering system (Pregitzer et al., 2002) and primary roots were classified into short and thin fibrous roots and longer and thicker pioneer roots (Polverigiani et al., 2011; Zadworny and Eissenstat, 2011). These two root clas- ses differ in their life expectancies and in their uptake and transport capacities (Zadworny and Eissenstat, 2011; Bagniewska-Zadworna et al., 2012), but it remains unknown if this classification also has consequences for root exudation rates in different soil layers.

Despite narratives of decreasing root exudation with increasing soil depth, quantitative information on in situ root exudation in the subsoils of mature forest stands is essentially absent. In our study we investigated fine root morphology and fine root exudation in a mature European beech forest to a soil depth of up to 130 cm in two growing seasons. The aim of the study was to detect adaptive responses of root exudation of beech to changing environmental conditions with increasing soil depth. We predicted that root exudation decreases with increasing root diameter and decreasing temperature, but increases with decreasing nutrient availability in subsoils, which could result in higher or lower root exudation at depth due to partly opposing effects of the environment.

Materials and Methods

Study site

Root exudates were collected at the Grinderwald site (52°14'019" N, 9°20'32" E; 100 m a.s.l.), northwest of Hannover, Germany, in four sampling campaigns between May 2014 and October 2015. At this site, a European beech (*Fagus sylvatica* L.) forest stand was established in 1916 (Forstamt Nienburg, 2010) in the center of the distribution range of European beech in the lowlands of NW Germany. At the time of the study, beech trees in the forest plantation had a basal area of 27 m^2 ha^{-1}, a closed canopy, and were of mature age (i.e., 100 years old). The parent materials for soil development were Pleistocene fluvioglacial sandy deposits from the penultimate (Saalian) ice age. The medium-to coarse-grained sandy deposits with low silt and clay content had a comparably low water storage capacity. The predominant soil type in the study area was an acidic (pH 3.4-4.5), sandy Dystric Cambisol (IUSS Working Group WRB, 2014) with a small AE horizon (2 cm; represents the topsoil). Subsoil was defined as the soil that is located below the A and E horizons (cf. IPCC, 2000), i.e. below 2 cm soil depth where the Bsw horizon started. The dominating humus form was a mormoder (classification according to Green et al., 1993).

Climate data were obtained from the German Meteorological Service (DWD) for a nearby climate station located in Nienburg (52°38'17" N, 9°12'30" E). Mean annual precipitation and temperature for the period 1947-2015 were 713 mm and 9.4° C (Table S1). Both study years had higher temperatures than the long-term average, with the warmer year 2014 surpassing the long-term mean by 1.7 °C and the long-term growing season mean by 0.9° C. Growing season precipitation was above average in 2014 (422 mm) and close to average in 2015 (371 mm).

Root exudate collection

In four sampling campaigns during the growing seasons 2014 and 2015 (i.e., May 2014, August 2014, June 2015, and October 2015) three soil pits of 1.5 m depth were excavated. The pits had a dis- tance of at least 3 m to the nearest mature beech tree. After excavation, root exudates were collected in three different depths, in the topsoil, the upper subsoil, and the lower subsoil. Since roots were not evenly distributed across the pit walls, we defined depth classes from which root exudates were collected. Specifically, the $subsoil_{40}$-depth class covered a depth range of 20-50 cm and the $subsoil_{100}$-depth class a depth range of 60-130 cm. In each soil pit,

root exudates were collected from three root strands (i.e., from the head wall of the soil pit and the two side walls) per soil depth class.

For the collection of root exudates in cuvettes filled with 2-mm diameter glass beads (*cf.* Phillips et al., 2008), root strands still attached to a mature tree were carefully extracted from the soil surface of the pit walls and all soil adhering to the root system was carefully removed with deionized water and fine forceps to main- tain the integrity of the root. Living root systems were then 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 $CaCl_2$). 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 solutions (i.e. no roots) were included as controls. Roots were allowed to equilibrate in the cuvette environment for 48 h before being flushed with dilute nutrient solution using a low-pressure vacuum. New nutrient solution was added and equilibrated for another 20 h. We collected these trap solutions containing exudates from each cuvette, filtered them through sterile syringe filters (GE Healthcare Life Sciences Whatman, Glass Microfiber Filters, Grade GF/F) and froze them at -20°C. Trap solutions were analyzed for dissolved organic C on a total organic carbon 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 (µmol C g^{-1} h^{-1}). Fine root biomass-depth relationships were established in a related earlier study by Meier et al. (unpublished results) in three similar mature European beech forest stands on the same Pleistocene fluvioglacial sandy deposits from the Saalian ice age, located nearby of the current study. Annual exudation C fluxes (in g C 10 cm^{-1} m^{-2} yr^{-1}) were estimated by multiplying the average mass-specific exudation flux for each individual soil pit with the average fine root biomass in each soil depth and multiplying daily exudation rates by the average length of the growing season of European beech in the northern part of Central Germany (225 days).

Root morphology

After root exudate collection root strands were clipped of the tree, immediately transported to the lab and stored at 6°C until processing. Fine root morphology (length, surface area, and diameter) was analyzed for all fine root samples by optical surface area measurement with a flat-bed scanner and the program WinRHIZO (Régent Instruments, QC, Canada).

Subsequently, root biomass was determined by drying (48 h, 70°C) and weighing. Specific root area (SRA, in $cm^2\ g^{-1}$), specific root length (SRL, in $m\ g^{-1}$), and root tissue density (in $mg\ cm^{-3}$) were calculated from these measurements.

Microbial biomass C, extractable organic C (EOC), and extractable N (ETN)

Soil samples were collected in June 2015 from three soil pits at each three different soil depths (horizontal collection at 5, 45, and 110 cm; n 1⁄4 3 per pit and soil depth class). The chloroform fumigation extraction (CFE) method (Vance et al., 1987) was used to determine microbial biomass carbon (C_{mic}). Briefly, chloroform fumigated (24 h) and non-fumigated samples with a fresh soil weight of 10 g were extracted with 40 ml of 0.025 M K_2SO_4 on a horizontal shaker at 250 rpm for 30 min and centrifuged at 4420 g for 30 min (Marhan et al., 2010). After the addition of 60 µl of 2 M HCl to the supernatants of each sample to remove potentially present inorganic C, organic C and total N were measured using a TOC-TNb Analyzer (Multi-N/C 2100S, Analytik Jena, Germany). Since only visible roots were removed prior to 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 is given as $\mu g\ C_{mic}\ g^{-1}$ DM. Extractable organic carbon (EOC) and extractable total nitrogen (ETN) were calculated from the values of the non-fumigated samples.

Phosphorus availability

The fraction of plant-available phosphorus according to Bowman and Cole (1978) was determined by resin bag extraction (anion exchange gel; Dowex 1 x 8-50; Dow Water & Process Solutions, USA). The resin was placed for 16 h in a suspension of 1 g field-moist soil material suspended in 30 ml water (Sibbesen, 1977). P was re-exchanged by 10% NaCl and 2% NaOH solutions and analyzed by color reaction with 5 mM hexaammonium heptamolybdate (Murphy and Riley, 1962) and photometric measurement at 712 nm against water (spectrophotometer; Libra S 21, Biochrom, UK). 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.

Soil fractionation and chemical composition

Air-dried and sieved (<2 mm) bulk soil collected in June 2013 from three soil pits at each three different soil depths (horizontal collection at 10, 35, and 110 cm; n = 3 per pit and soil depth class) were subjected to a combined density and particle size fractionation procedure according to Angst et al. (2016 b). As demonstrated by Angst et al. (2016 b), the combined fine silt and clay fraction dominated the soil organic carbon (SOC) storage at the Grinderwald site. Thus, we obtained the combined fine silt and clay fraction representing mineral associated OC and the particulate organic matter (POM).

In brief, 30 g of bulk soil were saturated with a sodium polytungstate (SPT) solution (TC Tungsten Compounds, Grub am Forst, Germany) with a density of 1.8 g cm^{-3}. Ultrasonication (600 J ml^{-1}) was used to break up soil aggregates; subsequently the floating light POM fraction was removed. The POM fraction was rinsed with deionized water until the electrical conductivity dropped below 5 µS, freeze-dried and stored for further analysis. The heavy mineral residue was rinsed with deionized water until the conductivity dropped below 50 µS and wet-sieved to separate all coarse fractions (20-2000 µm). The mineral soil smaller than 20 µm was subjected to sedimentation to obtain the combined fine silt and clay fraction (<6.3 µm), which was freeze-dried and stored for further analysis. The combined fine silt and clay fraction is referred to as 'clay fraction'.

The POM and clay fractions were analyzed for their chemical composition using solid-state ^{13}C CPMAS NMR spectroscopy on a Bruker DSX 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). The samples were spun in zircon oxide rotors around a magic angle at a speed of 6.8 kHz. The contact time was set to 1 ms. Due to the rather low C content in the clay fraction and very low total amounts of POM from 110 cm soil depth (*cf.* Angst et al., 2016b), only informative spectra of two samples for each fraction were obtained at that depth. To unravel the predominant compound classes of the SOM, we applied a molecular mixing model according to Nelson and Baldock (2005) based on the NMR data. The spectra were separated into seven integration areas, amide/ carboxyl (215-165 ppm), phenolic (165-145 ppm), aromatic (145- 110 ppm), di-O-alkyl (110-95 ppm), O-alkyl (95-60 ppm), N-alkyl/ methoxyl (60-45 ppm), and alkyl C (45-10 ppm). The molecular mixing model estimates the relative content of four components: carbohydrates, lignin, proteins, and lipids based on the signal intensity of the moieties in each of the seven integration regions.

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 $P \leq 0.05$ in most cases; in some cases significance at $P \leq 0.1$ is reported to indicate marginal significant differences. Means and standard errors were calculated from the mean of each three soil pits per study site, while samples taken from the three walls of a pit were treated as pseudo- replicates by averaging over them. The probability of a fit to a normal distribution was tested using a Shapiro-Wilk test ($P \leq 0.05$). Soil pit or study site means were compared by one-way analysis of variance (ANOVA) or by one-way Kruskal-Wallis single factor analyses of variance and non-parametric 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 soil depth 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.

Results

Soil depth and season effects on root exudation

Root exudation was significantly lower in the subsoil$_{100}$ than in the upper soil layers (topsoil and subsoil$_{40}$) at all four sampling dates (Fig. 1). The average mass-specific exudation rate varied between 33 and 82 µg C g^{-1} h^{-1} in the topsoil, between 25 and 62 µg C in the subsoil$_{40}$, and between 5 and 33 µg C in the subsoil$_{100}$. The average depth effect of subsoil$_{100}$ on root exudation was 82.4% (significant; Fig. 2). While a significant decrease occurred at all four sampling dates (and was similar for the relative decreases in exudation rates), the absolute decrease in root exudation rates with soil depth was higher in 2014 than in 2015 (decrease by 48-60 and 28-36 µg C g^{-1} h^{-1} in 2014 and 2015, respectively) due to higher root exudation rates in the topsoil in 2014 (75-82 and 33-42 µg C g^{-1} h^{-1} in 2014 and 2015, respectively; partly significant; Fig. 1). The difference between years was more pronounced than the difference between early and late seasons: root exudation did not differ significantly between early and late season in a given year in any soil layer. Both soil depth and sampling date had a significant effect on root exudation rates, but it responded stronger to soil depth than to sampling date (Table 1).

The average yearly C flux by root exudation decreased with increasing soil depth from 312 to 215 and 80 mg C $g^{-1}_{root\ mass}$ yr^{-1} and reached only about 26% in $subsoil_{100}$ as compared to the topsoil (difference significant; Table 2). Due to the exponential decrease in fine root biomass with soil depth from approximately 170 g 10 cm^{-1} m^{-2} in the topsoil to 65 g 10 cm^{-1} m^{-2} in the $subsoil_{40}$ and only 15 g 10 cm^{-1} m^{-2} in the deep $subsoil_{100}$ (*cf.* Meier et al., unpublished results), the depth effect on the annual C flux by root exudation in the forest stand multiplied: all soil depths differed significantly from each other, with exponential decreases in their annual root exudation flux from 52 g C 10 cm^{-1} m^{-2} yr^{-1} in the topsoil to 15 g C 10 cm^{-1} m^{-2} yr^{-1} in the upper subsoil and 1 g C 10 cm^{-1} m^{-2} yr^{-1} in the lower subsoil. Consequently, annual C flux by root exudation in the lower subsoil differed by more than a magnitude from the C flux in the upper soil layers.

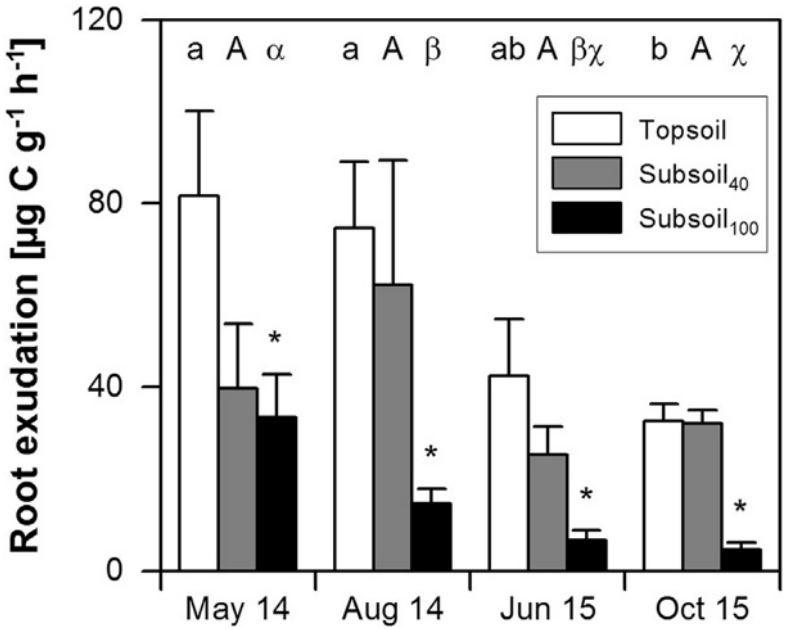

Fig. 1. Seasonal variation in root exudation rates at three soil depths in a European beech (*Fagus sylvatica* L.) forest from 2014 to 2015. Exudation values are means of three soil pits per sampling date. Asterisks indicate significant differences between subsoil and topsoil exudation. Different lower and upper case Latin and Greek letters indicate significant differences between months in their topsoil, $subsoil_{40}$, and $subsoil_{100}$ values, respectively. The $subsoil_{40}$-depth class covers a depth range of 20-50 cm, the $subsoil_{100}$-depth class a depth range of 60-130 cm.

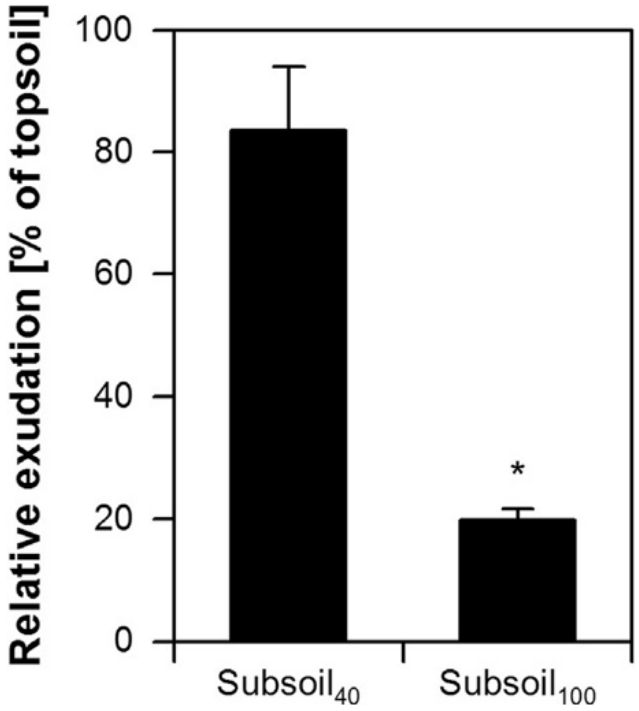

Fig. 2. Mean relative root exudation rates at two subsoil depths as percentage of topsoil root exudation rates in a European beech (*Fagus sylvatica* L.) forest. Exudation values are means of four sampling dates from May 2014 to October 2015. Asterisks indicate significant differences. The $subsoil_{40}$-depth class covers a depth range of 20-50 cm, the $subsoil_{100}$-depth class a depth range of 60-130 cm.

Table 1
Mixed effects models on the influence of soil depth (Depth) and sampling date (Season) on root exudation and root morphology in a European beech (*Fagus sylvatica* L.) forest. Given are *F*–test statistics values and probabilities of error *P*. Values used for mixed effects models are means of three samples per soil pit and soil depth. n.s. = not significant.

	Root exudation [μg C g^{-1} h^{-1}]		SRA [cm^2 g^{-1}]		Root diameter [mm]		Tissue density [mg cm^{-3}]	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Depth	22.7	<0.001	15.6	<0.001	9.9	0.001	6.6	0.006
Season	9.0	<0.001	2.3	n.s.	2.3	n.s.	1.5	n.s.
Depth x season	1.6	n.s.	2.3	0.07	1.1	n.s.	1.9	n.s.

Table 2
Estimated annual carbon flux by root exudation at three soil depths in a European beech (*Fagus sylvatica* L.) forest. Exudation rates are means of four sampling dates from May 2014 to October 2015. Different upper and lower case letters indicate significant differences between the soil depths. The $subsoil_{40}$–depth class covers a depth range of 20–50 cm, the $subsoil_{100}$–depth class a depth range of 60–130 cm. For comparability, fine root biomass (FRB) and the annual C flux by root exudation refer to a 10 cm-soil layer in each depth class.

Soil depth	Exudation rate [mg C g^{-1} yr^{-1}]	FRB [g m^{-2}]	Annual flux [g C m^{-2} yr^{-1}]
Topsoil	312 (64) ^{A}	168	52 (11) ^{a}
$Subsoil_{40}$	215 (43) ^{A}	65	15 (5) ^{b}
$Subsoil_{100}$	80 (35) ^{B}	15	1 (1) ^{c}

Soil depth and seasonal effects on root morphology

Specific root area (SRA) was significantly lower in the subsoil (155-187 cm^2 g^{-1}) than in the topsoil (258 cm^2 g^{-1}) across sampling dates (Fig. 3d). Season had no significant influence on SRA, since topsoil roots had the significant lowest SRA in the early season 2014 and the significant highest SRA in the early/mid-season 2015 (216 and 300 cm^2 g^{-1}; significant difference); while $subsoil_{40}$ roots had a significantly lower SRA in the early season 2014 than at the other sampling dates (106 vs. 181-245 cm^2 g^{-1}; Fig. 3a). The cross-effect of soil depth and sampling date was marginally significant (Table 1).

Both mean root diameter and root tissue density were significantly higher in the subsoil than in the topsoil across sampling dates (Fig. 3e and f). While increases in root tissue density with soil depth in principle occurred at all sampling dates, the increases were only significant in the early/mid-seasons 2014 and 2015 and not in the late seasons (Fig. 3c). There was no significant influence of season on root diameter or root tissue density (Table 1).

Relationships between root morphology and root exudation

Across seasons, the absolute exudation C flux per root sample was highly significantly influenced by the root mass inserted into the cuvettes, as well as by root length and root surface area in both the topsoil and $subsoil_{100}$ (Table S2). Absolute root exudation increased in the topsoil

by 0.5 µmol C h^{-1} with an increase in root mass by 0.1 g, but by only 0.1 µmol C h^{-1} in the $subsoil_{100}$ (Fig. S1a).
The absolute C flux was significantly influenced by tissue density (negative) and SRA (positive) in the early/mid-season 2014 and 2015, but there was no significant influence of morphology in the late seasons (Table S2). In the early season 2014, root exudation increased by 0.1 µmol C h^{-1} with a decrease in root tissue density by 100 mg cm^{-3}, while in early/mid-season 2015 it increased by only 0.04 µmol C h^{-1} (Fig. S1b).

Soil depth effect on microbial biomass and nutrient availability

The microbial biomass significantly decreased with soil depth from 56 µg C_{mic} g^{-1} in the topsoil to 13 µg C_{mic} g^{-1} in the $subsoil_{100}$ (decrease by 70%; Fig. 4). The amount of EOC decreased by even almost 9-fold from 133 µg g^{-1} in the topsoil to 16 µg g^{-1} in the $subsoil_{100}$ (Table 3). While ETN showed a similar decrease in N availability with increasing soil depth (10, 4, and 2 µg g^{-1} in the topsoil, $subsoil_{40}$, and $subsoil_{100}$, respectively), the amount of plant- available P was comparably high in the organic topsoil (14 µg g^{-1}) and low in both subsoil layers (0.7-1.3 µg g^{-1}).

Depth-dependent composition of particulate and mineral associated organic matter

While there was no pronounced difference in the chemical composition between topsoil and $subsoil_{40}$, the composition of the POM and clay fraction at $subsoil_{100}$ differed substantially from the topsoil fractions (Table 4). A significant decrease in the amount of proteins was detected for both fractions from the topsoil to the $subsoil_{100}$, and the amount of proteins was in all depth classes lower in the particulate (POM) than in the mineral-associated organic matter (clay). In an opposite trend, the amount of lignin increased from the topsoil to the subsoil100 (significant for the mineral-associated organic matter only). Carbohydrates and lipids did not consistently change with soil depth, but the amount of lipids was higher in the POM fraction.

Discussion

Rhizodeposition may represent a key biogeochemical process determining the amount and sink strength for C in forest subsoils, but, to the best of our knowledge, root C exudation in deep soil layers under mature forest trees has never been quantified before due to methodical constraints. In this study, we determined *in situ* root exudation in soil depths up to 130 cm and found a large decrease in mass-specific root exudation in the subsoil of the investigated European beech forest. Specifically, we proved that both the root mass- specific exudation activity (in µmol C $g^{-1}_{root\ mass}$ h^{-1}; decrease by -82%) as well as the soil-volume related exudation flux (in g C 10 cm^{-1} m^{-2} yr^{-1}; decrease by -98%) decreased significantly toward the deep subsoil to a fraction of the exudation C flux in the topsoil. The two exudation terms describe different functions: the former illustrates the specific activity of roots while the latter refers to the absolute C flux, which is also influenced by the fine root biomass in each soil depth. Accordingly, they prove that the decrease in exudation toward the subsoil was driven by a major decrease in specific exudation activity of fine roots and amplified by the accompanying exponential decrease in fine root biomass per soil volume.

Annual exudation flux in temperate forests

Root exudation rates in the topsoil of this study are of similar magnitude as the budgetary calculations for rhizodeposition con- ducted for a hardwood forest (Hubbard Brook Experimental Forest; dominated by American beech, sugar maple, and yellow birch) located on an acidic Spodosol on glacial deposits (80 g C 20 cm^{-1} m^{-2} yr^{-1}; Fahey et al., 2005). On nutrient-richer silty- loams, American beech and white oak (both ectomycorrhizal, ECM) exuded only about a third of this amount (26 g C 15 cm^{-1} m^{-2} yr^{-1}), but still three-fold more than sugar maple and tulip poplar (both arbuscular mycorrhizal, AM; 8 g C 15 cm^{-1} m^{-2} yr^{-1}; Yin et al., 2014). Loblolly pine (ECM) on an Alfisol with relatively high native fertility exuded 23 g C 15 cm^{-1} m^{-2} yr^{-1} (Phillips et al., 2011), despite the longer growing season of the evergreen in comparison to the deciduous tree species. The higher exudation rates in ECM than in AM trees were interpreted to reflect differences in N availability between these two major mycorrhizal as- sociation types, with the majority of soil N is contained in SOM rather than in mineral-associated C forms in ECM forests (Brzostek et al., 2014; Yin et al., 2014). Comparably low N availability mainly from organic N forms in the POM-dominated topsoil (Angst et al., 2016 b) of the investigated

acidic forest site may also explain the comparably high topsoil exudation rates of European beech, while the subsoils showed very low bulk OC contents, with clear dominance of mineral-associated OC (Angst et al., 2016 b).

Influence of root morphology and root system architecture on root exudation

In our study, beech roots in the subsoil had a significantly smaller specific root surface area and larger mean diameter and tissue density, i.e. they were thicker and sturdier in deep soil. Such a change in root morphology has been found to relate to changes in the metabolic activity of the roots. It has been demonstrated that roots of lower specific surface area or higher diameter with lower number of root tips have lower root respiration rates (Pregitzer et al., 1998; Polverigiani et al., 2011; Roumet et al., 2016) and a higher longevity (Wells and Eissenstat, 2001; Guo et al., 2008; Fan and Guo, 2010; De Graaff et al., 2013) than roots of higher specific surface area or lower diameter. Roots produced in subsoils often have an increased diameter (Wells et al., 2002) and longer lifespan (Wells et al., 2002; Guo et al., 2008), leading to decreased root respiration rates (Pregitzer et al., 1998). In other studies, root length and root branching intensity were positively correlated with root exudation (Xu and Juma, 1994; Groleau-Renaud et al., 1998; Darwent, 2003; Badri and Vivanco, 2009; Yin et al., 2013). Differences in root exudation in our study may, thus, partly be a consequence of a change in root morphology.
Tree root systems have a complex architecture and fine roots in different soil layers differ in their position in the branching hierarchy, with deep soil layers dominated by pioneer roots and upper soil layers by fibrous fine roots. Next to root morphology, the position in the branching hierarchy of a root system is also defining the functional status of roots (Pregitzer et al., 2002; Guo et al., 2008). Even within the first root order, heterorhizy (Noelle, 1910) is causing important consequences for overall functions: short and thin, fibrous roots with short lifespan mainly absorb nutrients and water while long, fast-growing pioneer roots with relatively long life expectancies mainly expand the root system horizontally and vertically and eventually become higher order roots (Polverigiani et al., 2011; Zadworny and Eissenstat, 2011). Several lines of evidences indicate that this classification may also result in functional differences in exudation rates between pioneer and fibrous roots: the primary function of pioneer roots is transport and not nutrient uptake which is facilitated by (i) the preferential formation of tracheary elements and more hypodermal layers with fewer passage cells (i.e. secondary growth) than in first-order fibrous roots (Zadworny and Eissenstat, 2011; Bagniewska-Zadworna et al.,

2012). This formation of a thicker, secondary periderm limits the metabolically active surface area for root exudation to the elongation zone immediately behind the root cap (Pineros et al., 2002). Pioneer roots (ii) grow preferentially in moist sections of soil (Polverigiani et al., 2011) and (iii) are less likely to be colonized by mycorrhizal and non-mycorrhizal fungi (Zadworny and Eissenstat, 2011) which limits their role in nutrient acquisition. Finally, pioneer roots (iv) receive differential investments of defenses (i.e. differ in their phenolic profile) than first-order fibrous roots which decreases their susceptibility to pathogen colonization (Emmett et al., 2014), but may also decrease the metabolic activity of the root surface. Thus, the observed shift from fibrous fine roots in the topsoil to pioneer roots in the deep subsoil in our study (cf. Fig. S2) may have caused functional differences between fine roots with different positions in the branching hierarchy of the root system and can be an explanation for the significant decrease in root exudation with increasing soil depth.

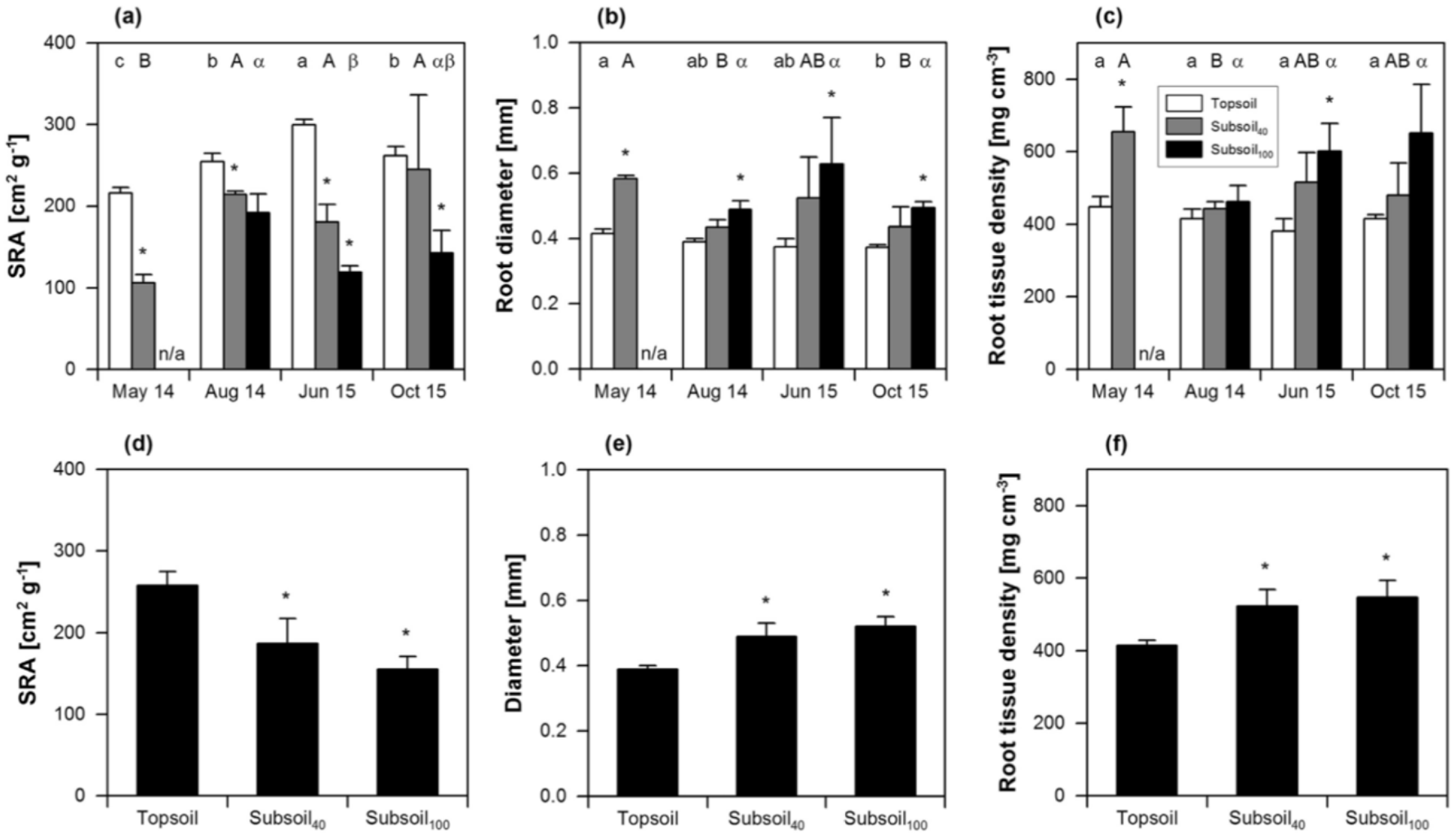

Fig. 3. Variation in **(a, d)** specific root area (SRA), **(b, e)** mean root diameter, and **(c, f)** root tissue density at three soil depths in a European beech (*Fagus sylvatica* L.) forest from May 2014 to October 2015. Morphology values are means of **(a, b, c)** three soil pits per sampling date and **(d, e, f)** four (100 cm: three) sampling dates. Asterisks indicate significant differences between subsoil and topsoil exudation. Different lower and upper case Latin and Greek letters indicate significant differences between months in their topsoil, $subsoil_{40}$ and $subsoil_{100}$ values, respectively. The $subsoil_{40}$–depth class covers a depth range of 20–50 cm, the $subsoil_{100}$–depth class a depth range of 60–130 cm.

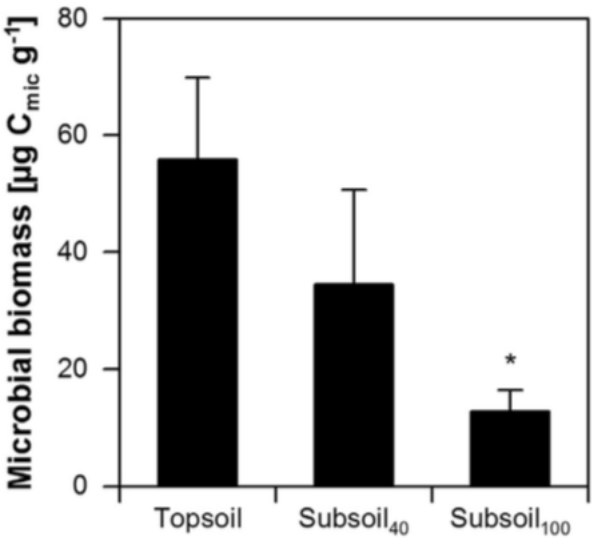

Fig. 4. Microbial biomass at three soil depths in a European beech (*Fagus sylvatica* L.) forest in June 2015. Values are means of three soil pits. Asterisks indicate significant differences between subsoil and topsoil microbial biomass. Samples for the $subsoil_{40}$–depth class were taken from 45 cm and samples for $subsoil_{100}$–depth class from 110 cm.

Changes in environmental conditions with soil depth

Important soil properties change with soil depth which can have an effect on root exudation: (i) bulk density is increasing, (ii) oxygen supply is decreasing, (iii) growing season temperature is decreasing and buffered against seasonal fluctuation, and (iv) energy (due to reduced input of fresh organic matter) and plant- available nutrients are increasingly limiting at depth (Schenk, 2005). However, both bulk density and oxygen supply do not explain root exudation rates in our study: it has been demonstrated that high mechanical impedance as it is the case in deep soil typically leads to an increase in mass-specific root exudation rates (Boeuf-Tremblay et al., 1995; Groleau-Renaud et al., 1998; Walker et al., 2003), which is in contrast to the results of our study. Similarly, hypoxia caused by decreasing oxygen contents is thought to lead to a shift from aerobic respiration to the fermentation of carbohydrates and increased exudation of the accumulated products into the rhizosphere (Xia and Roberts, 1994; Neumann and Römheld, 2007), which is also contradicting our results.

The observed decrease in root exudation could have been a result of decreasing temperature with soil depth: the passive part of exudation is likely to be affected by rhizosphere temperature, since the speed of the diffusion process and membrane stability are temperature-dependent (Neumann and Römheld, 2007). Active exudation processes that depend on metabolic energy

can also be limited at lower temperatures (Neumann and Römheld, 2007). Accordingly, warming enhanced specific root C exudation in temperate deciduous and subalpine coniferous forests (Boone et al., 1998; Yin et al., 2013; Zhang et al., 2016). But the vertical gradient in soil temperature was not strong at our study site (decrease of annual soil temperature from 8.9 ºC at 30 cm soil depth to 8.4 ºC at 90 cm; Preusser et al., personal communication) and, thus, may have limited explanatory power for the exponential decrease in root exudation we observed. In other studies, exudation rates of temperate deciduous tree species exhibited seasonal variation coincident with patterns of soil temperature, with the highest exudation rates occurring when soil temperature was high (Yin et al., 2014). The measured root exudation of the investigated beech trees of our study demonstrated a subordinate seasonal and a dominating depth effect (Table 1), despite maximum temperature amplitude of *c.* 10 ºC across the sampling dates (Table S1). In fact, root exudation was not significantly influenced by air temperature, since it was slightly higher in both the coldest and the warmest sampling date than at the other two sampling dates with intermediate temperatures (difference not significant; Fig. 1), which seems to contradict a dominant temperature effect on root exudation in our study. Girdling and pulse labelling studies already indicated that, next to an influence of soil temperature, exudate fluxes are mainly driven by carbohydrate source-sink relationships in the plant (Phillips et al., 2008; Kaiser et al., 2010).

Since root exudation is generally increased in response to nutrient deficiency in the topsoil (Neumann and Römheld, 1999; Phillips et al., 2011; Yin et al., 2014) due to the active secretion of specific carboxylates via anion channels (Jones et al., 2004), the same can be expected with vertical gradients in nutrient avail- ability from topsoil to subsoil. Again, this expectation is contrasting the result of our study, since we found lower root exudation in the nutrient-poor subsoil. It has been shown that the abundance of microorganisms affects rhizodeposition (Meharg and Killham, 1991; Neumann and Römheld, 1999; Fransson and Johansson, 2010; Meier et al., 2013) which would lead to enhanced root exudation in the topsoil with generally higher microbial abundance and activity in comparison to the SOC-poor subsoil (Fig. 4; Fierer et al., 2003; Fang and Moncrieff, 2005).

Table 3
Soil moisture, extractable organic carbon (EOC), extractable total nitrogen (ETN), and plant-available phosphorus (P_{resin}) at three soil depths in a European beech (*Fagus sylvatica* L.) forest. Different lower and upper case letters indicate significant differences between the soil depths. The soil samples for the $subsoil_{40}$–depth class were taken at 45 cm depth, soil samples for the $subsoil_{100}$–depth class at 110 cm depth.

Soil depth	Soil moisture [% SWC, w/w]	EOC [$\mu g\ g^{-1}$]	ETN [$\mu g\ g^{-1}$]	C:N [$g\ g^{-1}$]	P_{resin} [$\mu g\ g^{-1}$]
Topsoil	32.5 (3.3) ^{a}	133.2 (14.8) ^{A}	9.9 (1.6) ^{a}	13.8 (1.3) ^{b}	13.7 (1.8) ^{a}
$Subsoil_{40}$	7.0 (1.3) ^{b}	86.5 (15.7) ^{A}	4.4 (0.9) ^{b}	20.4 (1.9) ^{a}	0.7 (0.2) ^{b}
$Subsoil_{100}$	7.0 (3.5) ^{b}	15.5 (6.2) ^{B}	1.9 (0.7) ^{b}	8.1 (0.7) ^{c}	1.3 (0.6) ^{b}

Table 4
Relative amounts (%) of carbohydrates, proteins, lignin, and lipids in two soil organic matter (SOM) fractions representing mineral-associated (clay) and particulate organic matter (POM) at three soil depths in a European beech (*Fagus sylvatica* L.) forest. Different lower and upper case letters indicate significant differences between the soil depths. The soil samples for the $subsoil_{40}$–depth class were taken at 35 cm depth, soil samples for the $subsoil_{100}$–depth class at 110 cm depth.

Soil depth	Carbohydrates	Proteins	Lignin	Lipids
Clay				
Topsoil	19.3 (2.2) ^{a}	17.2 (0.6) ^{A}	28.3 (1.7) ^{b}	33.6 (4.5) ^{A}
$Subsoil_{40}$	17.6 (1.6) ^{a}	14.1 (2.5) ^{AB}	26.8 (1.1) ^{ab}	37.2 (3.9) ^{A}
$Subsoil_{100}$	14.6 (0.3) ^{a}	6.3 (1.4) ^{B}	35.6 (6.2) ^{a}	30.2 (4.1) ^{A}
POM				
Topsoil	11.7 (1.3) ^{a}	7.5 (1.3) ^{A}	28.5 (1.5) ^{a}	53.1 (3.8) ^{A}
$Subsoil_{40}$	12.2 (2.3) ^{a}	3.4 (2.3) ^{AB}	30.0 (3.2) ^{a}	54.7 (6.1) ^{A}
$Subsoil_{100}$	12.8 (1.4) ^{a}	0.1 (0.08) ^{B}	43.7 (1.7) ^{a}	39.0 (0.6) ^{A}

Priming of microbes to decompose SOC by root exudation

Roots interact with rhizosphere microbes to stimulate the decomposition of less bioavailable SOC by the addition of a labile C source via rhizodeposition (priming effect; Kuzyakov et al., 2000). It has been found that priming effects were smaller in soil material from greater depths (relatively low total soil C and low labile:stable C ratio) compared to material from surface layers (relatively high total soil C and high labile:stable C ratio) and there are reports that exudation initially even suppresses SOC decomposition in subsoil, i.e. induced a negative priming effect, since microbes preferentially consumed exudate C (Salomé et al., 2010; De Graaff et al., 2014; Hafner et al., 2014). Rhizosphere priming effects may be greatest in (top)soils with N mostly being present in form of organic N (Drake et al., 2013), while in most subsoils, recalcitrance of specific chemical compounds leading to the stabilization of SOM is less important (Schöning and Kögel-Knabner, 2006; Fontaine et al., 2007; Kemmitt et al., 2008; Marschner et al., 2008; Salomé et al., 2010; Sanaullah et al., 2011), and a relatively high mineral nitrogen content and low C:N ratio (Table 3; Jenkinson et al., 2008) may diminish the importance of priming effects. For the study site, a clear dominance of mineral-associated SOM at greater soil depths was found (Angst et al., 2016 b), while the topsoil had a considerable amount of OC stored as POM. Vertical gradients in soil C availability may also influence the structure and function of soil microbial communities (Fierer et al., 2003; Goberna et al., 2005; Kramer et al., 2013; Herold et al., 2014; Delgado-Baquerizo et al., 2016), causing differences in their response to additions of labile C and subsequent priming effects. It has been

shown for an Eutric Cambisol, that fast-growing microbial communities in the comparably C-rich subsoil preferentially used simple molecules of the types usually found in root exudates (amino and organic acids) while topsoil microbial communities were adapted to use slightly more complex molecules such as polymers and disaccharides (Salomé et al., 2010). Microbes in deep soil are mainly energy- limited due to a lack of fresh organic C, making the decomposition of less bioavailable SOC unviable to sustain long-term biological activity (energetic barrier; Fontaine et al., 2007). Accordingly, responses to an increase in exudation in the subsoil may differ greatly, from stimulation of SOC decomposition in C-rich subsoil patches (Fontaine et al., 2007) to no effect or even suppression in C- poor subsoils (this study site: Wordell-Dietrich et al., 2016; De Graaff et al., 2014). The sharp decrease in protein C in both the particulate and mineral-associated fraction at 110 cm soil depth might point to an exhausted organic N pool, although the lower C/N ratios indicate a greater importance of microbial residues in the deeper subsoil (Angst et al., 2016 a). Interestingly, all other compounds, carbohydrates, lignin, and lipids, do not show significant differences between the top- and the subsoil except for the lignin content of the clay fraction. Thus, exudation seems not only be triggered by the composition of the SOC but the sheer abundance of OC. Especially in subsoils, the more heterogeneous spatial distribution of available OC compared to topsoils (Rumpel and Kögel-Knabner, 2011) might affect exudation. This has also consequences for the carbon invested by plants into root exudation and may lead to a restriction of the active part of root exudation and limitation of the C loss to basal root exudation which is seeping out of the root and which the plant cannot control. Such strategy, where individual root strands of a root system respond to the turnaround of the priming effect, is in accordance with the optimization of carbon-use efficiency at the whole-plant level, because root exudation is increased at soil spots where higher amounts of N and other nutrients can be mined by microbes, but it is curtailed where resources are poor in supply, thus maximizing overall tree productivity.

Conclusions

We conclude that mature beech reveals adaptive responses of the root system to subsoil conditions, in particular a decrease in specific root area and an apparent change in root C exudation patterns with increasing soil depth. Decreasing availability of mineral-associated SOC and organic N may be the main trigger for the shift in the morphology and function of primary roots to larger- diameter, pioneer roots scavenging for resources. This shift in root morphology is

accompanied by largely decreased specific root exudation rates which may be reduced to the level of basal exudation in deep soil layers. We hypothesize that the effect of root exudation on soil C decomposition in subsoil differs vastly from the response in the topsoil with priming of microbes being subordinate, which makes enhanced C investment into root exudation in C- poor subsoil dominated by mineral-associated C unviable for trees. Such a strategy, where root exudation is enhanced in SOM-rich soil environments but decreased in other soil layers, is in accordance with the optimization of carbon-use efficiency at the whole-plant level. This would also have consequences for predictions on the C sink strength of forests and the potential loss of ancient buried carbon (Fontaine et al., 2007), since increased C allocation to roots and deeper rooting under elevated CO_2 (Iversen, 2010) or with land-use change e.g. from agricultural fields to forests (Guo and Gifford, 2002; Wright et al., 2007; Follett et al., 2009) may not lead to enhanced root exudation and stimulation of microbial decomposition of recalcitrant C in subsoils.

Acknowledgements

The authors would like to thank Venket Raghavan for support with lab analyses. We thank Bernd Marschner and Stefanie Heinze for coordination of the research unit 'The Forgotten Part of Carbon Cycling: Organic Matter Storage and Turnover in Subsoils (SUB- SOM)' (funded by Deutsche Forschungsgemeinschaft DFG, FOR1806, MA 1830/14-1; subprojects: KA 1590/11-1, LE 762/15-1, MU 3021/4-1) which this study was associated to.

Appendix A. Supplementary data

Supplementary data related to this article can be found at dx.doi.org/10.1016/j.soilbio.2017.01.006.

Supplement

Table S1 Meteorological data for the days of exudate collection and during the study years. Values are averages of air temperature at 2 m height (T), minimum temperature at the soil surface (T_{Min}), relative air humidity (RH), and sums of precipitation and sunshine duration. Long-term averages refer to the period 1947-2015 (T_{Min}: 1962-2015; Sunshine duration: 1974-2015). GS = growing season.

Date	T [°C]	T_{Min} [°C]	Precip. [mm]	RH [%]	Sunshine dur. [h]
01-02/05/14	9.8	5.0	5.9	81.6	0.3
07-08/08/14	19.4	11.4	3.4	78.5	12.0
25-26/06/15	18.3	11.5	5.2	69.0	6.6
05-06/10/15	13.3	7.0	7.7	85.7	3.7
2014 (GS)	15.5 (1.2)	8.3 (1.2)	422	78.0 (1.0)	1108
2015 (GS)	14.6 (1.7)	6.7 (2.0)	371	72.4 (2.2)	1177
2014	11.1 (1.6)	4.9 (1.3)	654	81.0 (1.4)	1616
2015	10.4 (1.6)	4.0 (1.4)	733	78.5 (2.2)	1623
Mean GS	14.6 (0.1)	8.0 (0.1)	374 (9)	74.3 (0.3)	989 (26)
Mean annual	9.4 (0.1)	4.0 (0.1)	713 (15)	79.1 (0.2)	1364 (30)

Table S2 Correlations of absolute root exudation (C efflux in µmol C h^{-1}) at three soil depths in a European beech (*Fagus sylvatica* L.) forest with root morphology. Shown are Pearson correlation coefficients and significance levels. Significant correlations at $P \leq 0.1, \leq 0.5, \leq 0.01$ and ≤ 0.001 are indicated by (*), *, **, and ***, respectively. Values used for correlation analyses are the averages of three samples per soil pit. The $subsoil_{40}$–depth class covers a depth range of 20-50 cm, the $subsoil_{100}$–depth class a depth range of 60-130 cm. n/a = not applicable.

Response	Explanatory variable	Soil depth [cm]	Root mass [g]	Root length [cm]	Root surface area [cm^2]	SRA [$cm^2\ g^{-1}$]	Diameter [mm]	Tissue density [$mg\ cm^{-3}$]
	Soil depth							
Root exudation	Topsoil	n/a	**0.77 ****	**0.57(*)**	**0.65 ***	**-0.53 (*)**	0.46	0.23
	$Subsoil_{40}$	-0.19	0.13	0.14	0.41	-0.23	0.32	-0.18
	$Subsoil_{100}$	-0.42	**0.68 ***	**0.82 ****	**0.85 ****	-0.01	-0.03	-0.07
	Season							
Root exudation	May 14	**-0.76 ***	-0.43	0.76	0.38	**0.96***	-0.81	**-0.96 ***
	Aug 14	**-0.59 (*)**	0.20	0.51	0.41	0.45	-0.55	-0.15
	Jun 15	**-0.76 ***	-0.21	0.37	0.52	**0.66 (*)**	0.02	**-0.91 *****
	Oct 15	-0.48	0.56	0.23	0.45	-0.22	0.26	0.04

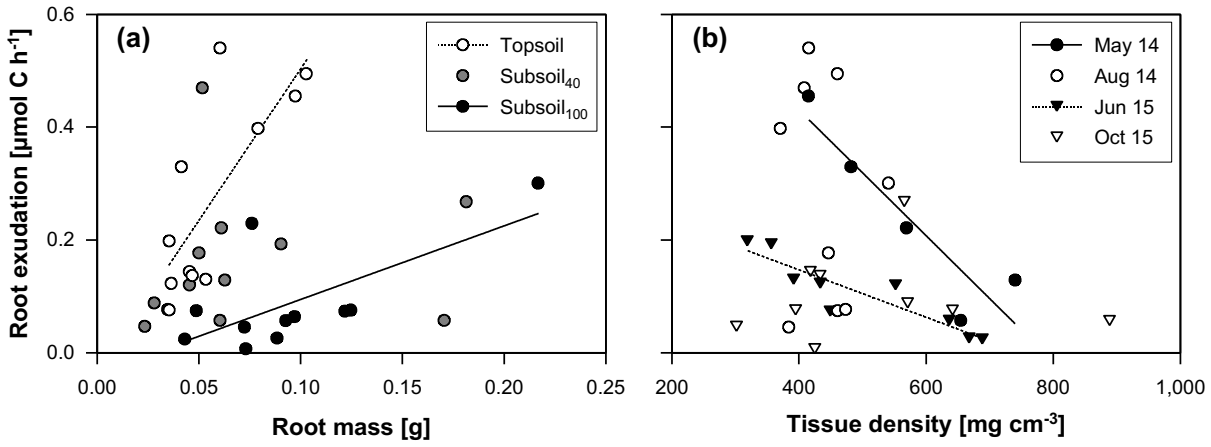

Figure S1 Correlations between absolute root exudation (C efflux) and **(a)** root mass or **(b)**average root tissue density at three soil depths in a European beech (*Fagus sylvatica* L.) forest from May 2014 to October 2015 (root mass: topsoil: y=-0.03+5.4x, R^2=0.59, P=0.003; $Subsoil_{100}$: y=-0.04+1.3x, R^2=0.46, P=0.01; tissue density: May 14: y=0.9-0.001x, R^2=0.84, P=0.01; Jun 15: y=0.3-0.0004x, R^2=0.82, P=0.001). Values used for correlation analyses are means of three samples per soil pit. The $subsoil_{40}$–depth class covers a depth range of 20-50 cm, the $subsoil_{100}$–depth class a depth range of 60-130 cm.

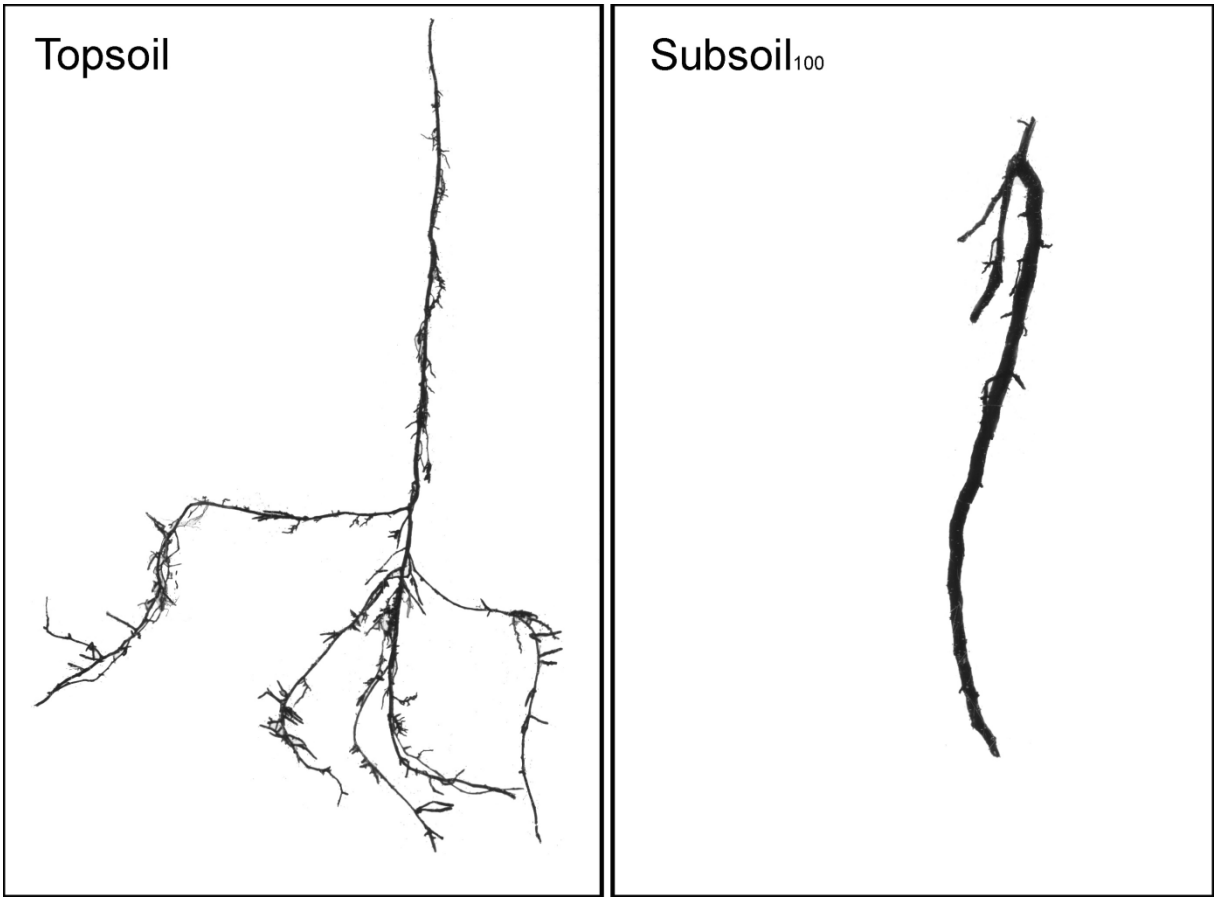

Figure S2 Root morphology of an example fibrous root strand in the topsoil and a pioneer root strand in the subsoil$_{100}$in a European beech (*Fagus sylvatica* L.) forest stand. The subsoil$_{100}$–depth class covers a depth range of 60-130 cm.

CHAPTER 3

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ROOT EXUDATION OF MATURE BEECH FORESTS ACROSS A NUTRIENT AVAILABILITY GRADIENT: THE ROLE OF ROOT MORPHOLOGY AND FUNGAL ACTIVITY

Ina Christin Meier · Timo Tückmantel · Julian Heitkötter · Karolin Müller · Sebastian Preusser · Thomas J. Wrobel · Ellen Kandeler · Bernd Marschner · Christoph Leuschner

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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

strongly influenced by the amount of avail- able photosynthates and thus depends on controlling 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-ß 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 activ- ity 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 rhizo- sphere 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 factors such as photosynthetically active radiation, atmospheric CO2 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

(Tückmantelet 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 excava- tion 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 through sterile syringe filters (pore size 0.7 µm, Whatman glass microfiber filters, grade GF/F; GE Healthcare Bio-Sciences, Pitts- burgh, 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).

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	Geol. epoch	Longitude [E]	Latitude [N]	Altitude [m a.s.l.]	Prec. [mm]	Temp [°C]	Assoc.	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 values, 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}
	+s.e.	0.03	0.03	0.01	0.08	0.07	0.05
	-s.e.	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}
	s.e.	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}
	s.e.	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}
	s.e.	0.5	0.2	0.1	0.3	0.1	0.5
Microbial activity							
(Hemi-)Cellulases activity	mean	0.53 ^{BC}	2.23 ^{A}	1.46 ^{AB}	0.80 ^{BCD}	0.49 ^{C}	0.14 ^{D}
[µg C g_{soil}^{-1} h^{-1}]	s.e.	0.22	0.34	0.84	0.42	0.03	0.01
NAG activity	mean	0.58 ^{abc}	1.70 ^{a}	1.33 ^{abc}	0.69 ^{abc}	1.04 ^{b}	0.43 ^{c}
[µg N g_{soil}^{-1} h^{-1}]	s.e.	0.40	0.21	0.99	0.39	0.15	0.04

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-b-glucosaminidase; n/d, not determined; SOC, soil organic carbon; SON, soil organic nitrogen.

1Based on the quantity of microbial biomarkers (Angst et al., 2018).

2Based on d13C phospholipid fatty acid analyses (Preusser et al., 2017).

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 x 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 (x40). 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 x 135 cm, which was equally divided into six grid cells of 45 x 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 μg 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; ß-glucosidase, BG; ß-xylosidase, BX; ß-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, Switzer- land) 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 ± 7 μgC 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 μgC g^{-1} h^{-1}), intermediate values on sand (31 μgC g^{-1} h^{-1}) and the significantly highest exudation rates in the topsoil on glacial deposits (65 μgC 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 (4–5gC 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 μgC g^{-1} h^{-1} in May

and August 2014, respectively, but decreased to 22 ± 5 µgC 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 carbona- ceous substrates was more variable across sampling dates than the top rank for the glacial deposits. Root exudation rates on lime-stone 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 ± 1m g^{-1}) and high values on glacial deposits (23 ± 2m 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 ± 1m g^{-1}) and increased towards June 2015 (23 ± 2m 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).

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).

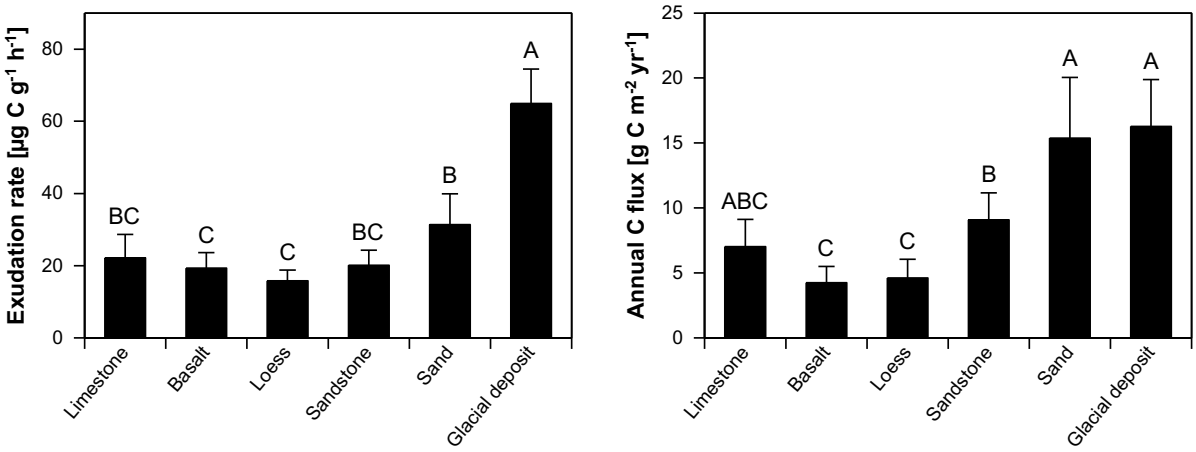

Fig. 1 Means + SE of (a) root exudation rates and (b) estimated annual carbon (C) flux from root exudation in the topsoil of European beech forests on six different bedrock types (n = 3 pits and n = 3 dates per substrate). Different upper-case letters indicate significant differences between the substrates. 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 (<1mm 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
Season	4.2	0.02	31.8	<0.001
Substrate x season	3.8	0.002	2.3	0.03

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

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 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.

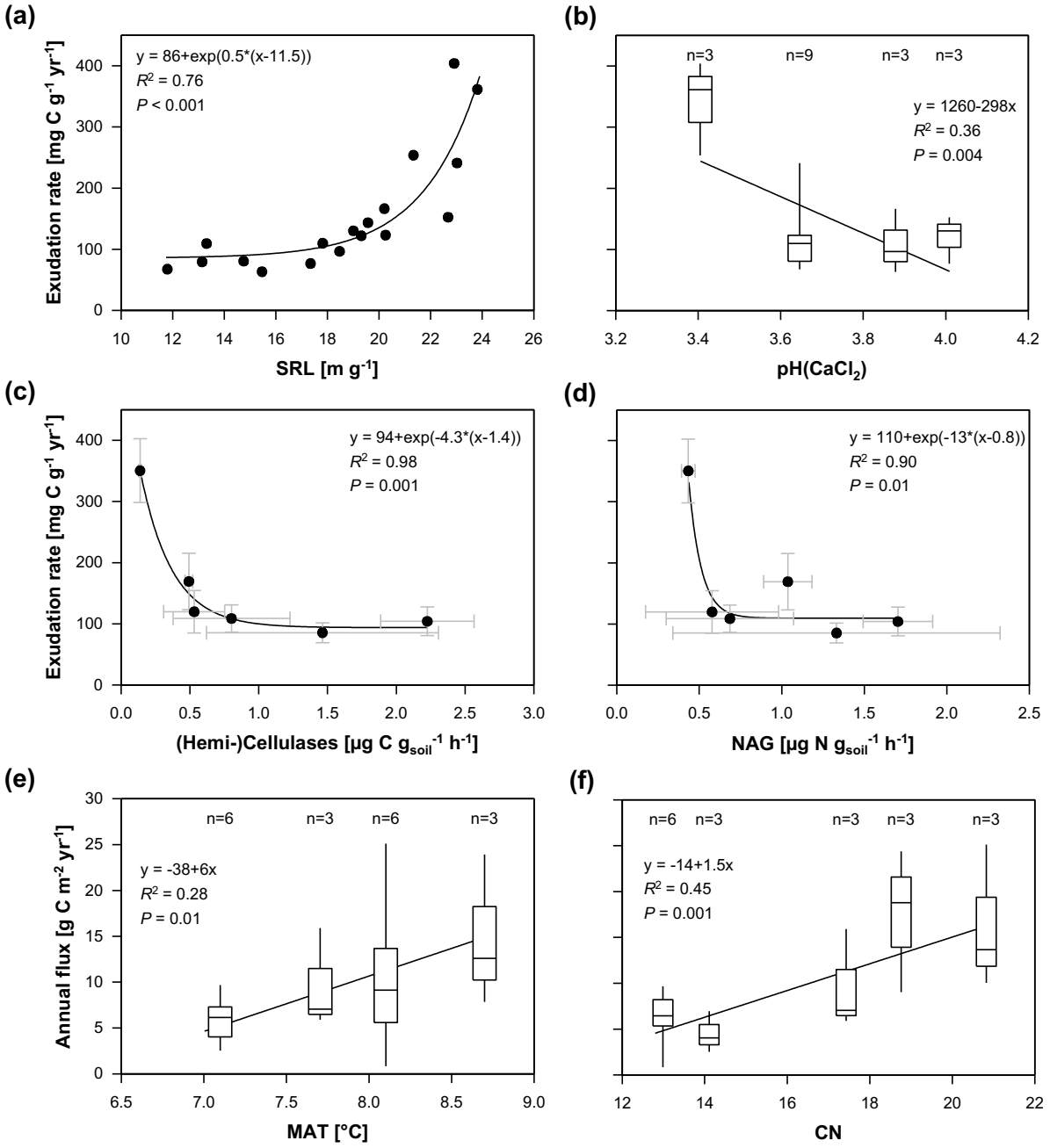

Fig. 2 Relationship of root exudation with (a) specific root length (SRL), (b) pH (CaCl2), (c) (hemi-)cellulase (a-glucosidase + b-xylosidase + bcellobiosidase) activity, (d) N-acetyl-b-glucosaminidase (NAG) activity, (e) mean annual temperature (MAT) and (f) soil carbon : nitrogen (C : N) in the topsoil 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 (<1mm in diameter) in the organic layer and 0–10 cm layer.

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 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).

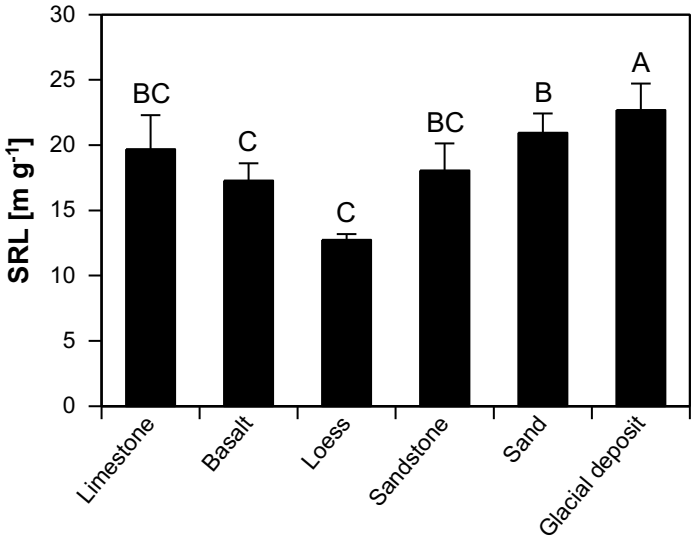

Fig. 3 Means + SE of specific root length (SRL) in the topsoil of European beech forests on six different bedrock types (n = 3 pits and n = 3 dates per substrate). Different upper-case letters indicate significant differences between the substrates.

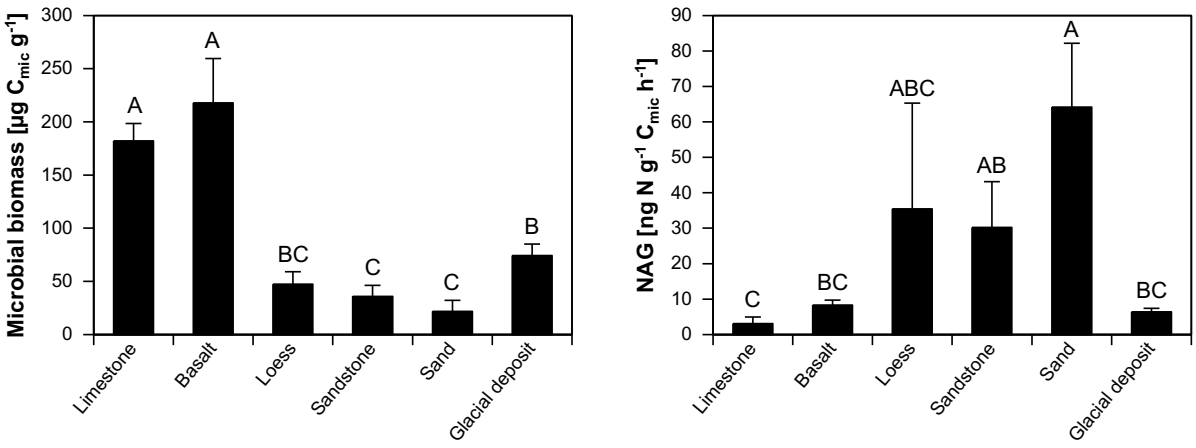

Fig. 4 Means + SE of (a) microbial biomass carbon (C) and (b) specific N-acetyl-b-glucosaminidase (NAG) activity per microbial biomass C in the topsoil of European beech forests on six different bedrock types (n = 4 pits per substrate). Study sites were sampled in June 2013 (glacial deposits) and May 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 (CaCl2), C : N and N-acetyl-b-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					
Y	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

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 µg 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 µg 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 µg 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.

Acknowledgements

Our study profited from its association to the research unit FOR 1806 'The Forgotten Part of Carbon Cycling: Organic Matter Storage and Turnover in Subsoils (SUBSOM)', funded by Deutsche Forschungsgemeinschaft (DFG). The support by coordinator Stefanie Heinze is gratefully acknowledged. We thank Kristina Kirfel for the supply of root biomass data and Mirjam Helfrich and Axel Don for the supply of SOC and SON data.

Supplement

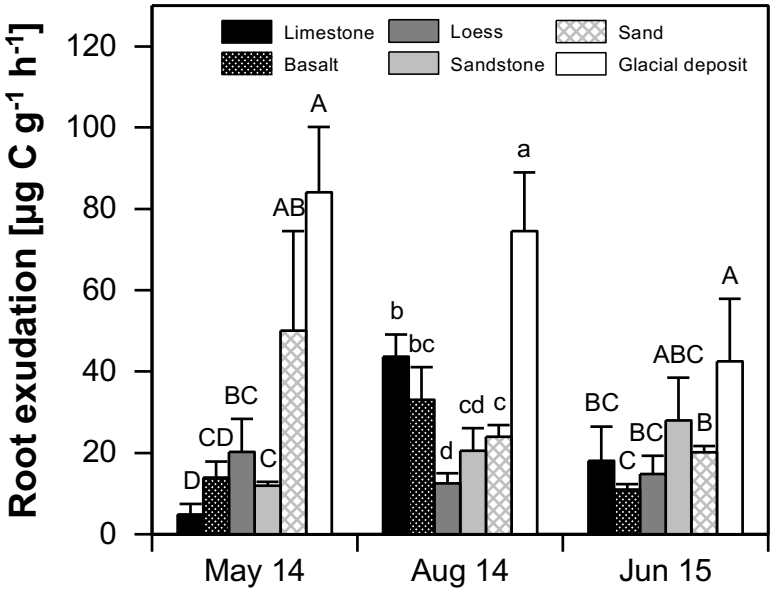

Fig. S1 Seasonal variation in root exudation rates in the topsoil of European beech (*Fagus sylvatica* L.) forests located on six different bedrock types between 2014 and 2015 (n = three pits per substrate). Different upper- and lower-case letters indicate significant differences between the substrates.

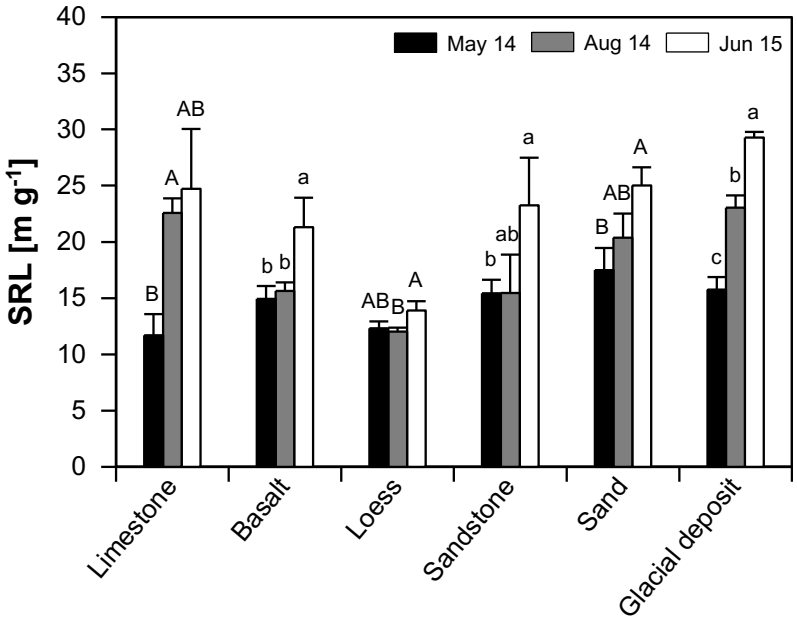

Fig. S2 Seasonal variation in specific root length (SRL) in the topsoil of European beech (*Fagus sylvatica* L.) forests located on six different geological substrates between 2014 and 2015 (n = three pits per geological substrate). Different upper- and lower-case letters indicate significant differences between the sampling dates.

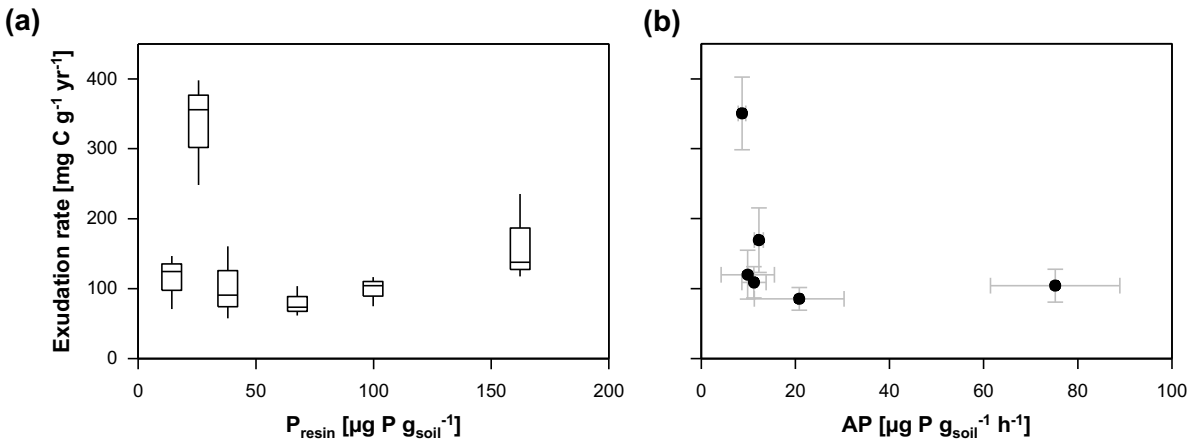

Fig. S3 Relationship of root exudation with **(a)** plant-available P (P_{resin}) and **(b)** alkaline phosphatase (AP) activity in the topsoil of European beech (*Fagus sylvatica* L.) forests on six different bedrock types. Shown are pit means for exudation (n = three samples and dates per substrate) and site means for enzyme activities (n = four pits per substrate). Root exudation was sampled from May 2014 to June 2015; enzyme activities were sampled in June 2013 (glacial deposits) and May 2014 (all other substrates).

Table S1 Estimated annual carbon flux with root exudation in the topsoil of European beech (*Fagus sylvatica* L.) forests on six different bedrock types

Substrate	Exudation rate [mg C g^{-1} yr^{-1}]	FRB [g m^{-2}]	Annual flux [g C m^{-2} yr^{-1}]
Limestone	120 (35) ^{BC}	74 (5) ^{b}	7 (2) ^{ABC}
Basalt	104 (23) ^{C}	53 (15) ^{c}	4 (1) ^{C}
Loess	85 (16) ^{C}	62 (24) ^{bc}	5 (1) ^{C}
Sandstone	109 (22) ^{BC}	103 (8) ^{a}	9 (2) ^{B}
Sand	169 (46) ^{B}	110 (8) ^{a}	15 (5) ^{A}
Glacial deposit	350 (52) ^{A}	64 (19) ^{b}	16 (4) ^{A}

Exudation rates are means of three sampling dates from May 2014 to June 2015 (n = three pits and dates per substrate). Different upper- and lower-case letters indicate significant differences between the substrates.

For comparability among substrates, FRB (<1 mm) and the annual C flux by root exudation refer to the organic layer and top mineral soil (0-10 cm soil depth).

CHAPTER 4

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TEMPERATURE EFFECTS ON ROOT EXUDATION OF MATURE BEECH (FAGUS SYLVATICA L.) FORESTS ALONG AN ELEVATION GRADIENT

Christoph Leuschner[1,3] · Timo Tückmantel[1] · Ina Christin Meier[1,2]

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Abstract

Aims: Root exudation may have a large impact on soil biological activity and nutrient cycling. Recent advances in *in situ*-measurement techniques have enabled deeper insights into the impact of tree root exudation on rhizosphere processes, but the abiotic and biotic controls of exudation rate remain poorly understood. We explored the temperature dependence of root exudation in mature beech (*Fagus sylvatica* L.) trees.

Methods: We measured fine root exudation in seven beech forests along an elevational gradient (310-800 m a.s.l.) and related carbon (C)-flux rates to mean daily temperature, actual precipitation, mean summer temperature (MST) and precipitation (MAP), soil moisture (SWC), and stand structure.

Results: Average mass-specific exudation (averaged over all sampling dates) ranged from 12.2 µg C g^{-1} h^{-1} to 21.6 µg C g^{-1} h^{-1} with lowest rates measured at highest elevations and peak rates at mid-elevation (490 m). Regression analyses showed a highly significant positive effect of site-specific daily air and soil temperature on exudation rates ($p < 0.01$) with an average increase by 2 µg C g^{-1} h^{-1} per 1 °C-temperature increase, while the relation to mean summer or annual temperature and mean temperature of the measuring year was less tight. Exudation decreased with increases in mean annual precipitation and soil moisture, and higher specific root area and stem density.

Conclusions: The root exudation rate of beech trees roughly triples between 10 °C and 20 °C mean daily temperature, evidencing a large temperature influence on root-borne C flux to the soil.

Keywords: air temperature, European beech, fine root morphology, mass-specific exudation, precipitation, soil moisture, soil temperature

Introduction

Climate warming affects biogeochemical processes and the carbon (C) cycle of forests through various direct and indirect interactions, notably temperature effects on photosynthesis, plant respiration, soil organic matter (SOM) decomposition, nutrient diffusion in soil, and root nutrient uptake kinetics, and the abundance and activity of mycorrhizal partners (Norby et al. 2007; Yin et al. 2013b). While much experimental research with trees has addressed warming effects on photosynthesis, respiration, belowground C allocation and fine root dynamics (Bai et al. 2010; Graham et al. 2014; Liang et al. 2013; Majdi and Ohrvik 2004), and soil microbial activity and SOM decomposition (von Lützow and Kögel-Knabner 2009), much less is known about the temperature dependence of rhizodeposition, i.e. the loss of carbon from roots (Yin et al. 2013b). Rhizodeposition, which includes exudation (the leakage of soluble organic compounds from living root cells), the secretion of mucilage and other organic substances, and the shedding of dead cells and tissues from roots, represents a net flow of C from roots to the soil which can have profound effects on C and nutrient cycling in forest ecosystems (Jones et al. 2009). Root exudation is thought to be a key mediator in plant-soil interactions that influences the composition and activity of soil microbial communities and thus soil enzyme activity, SOM decomposition and nutrient cycling (Cheng et al. 2014; Fransson and Johansson 2010; Gougherty et al. 2018; Jones et al. 2004; Pausch and Kuzyakov 2017). Estimates of the size of the C flux with root exudation in forest ecosystems vary widely from a few percent up to 21 % of net primary productivity (Badri and Vivanco 2009; Jones et al. 2004; Kannenberg and Phillips 2017; Meier et al. 2020; Pinton et al. 2007), or up to a third of the photosynthetic carbon gain of tree saplings (Liese et al. 2018). It is not well understood how an increase in temperature influences exudation rate, as this C flux is dependent on a multitude of biotic and abiotic factors and is rarely quantified under field conditions (Yin et al. 2013). In fact, it may represent the most uncertain part of the C cycle in ecosystems, especially in mature forests (Price et al. 2012).

Biotic factors influencing root exudation are carbohydrate availability and allocation to roots, plant phenology and development stage, root morphology (notably specific root length, SRL), the type of mycorrhizal fungi, and the presence of pathogenic soil microbes (Kuzyakov 2002; Meier et al. 2013; Neumann and Römheld 2007; Tückmantel et al. 2017) as well as root membrane conductivity. Abiotic factors that have been found to influence root exudation rate include radiation through its influence on photosynthesis (Nakayama and Tateno 2018), deficiency of nutrient elements such as phosphorus (P) and nitrogen (N) (Meier et al. 2020; Yin et al. 2014), soil moisture (Brunn et al. 2022; Jakoby et al. 2020; Preece et al. 2018;), and

temperature (Yin et al., 2013a). The majority of root exudates are believed to be passively lost from the root, thus being proportional to the root – soil solute concentration gradient (Jones et al. 2004). This suggests a possible influence of microorganism absorption on exudation rate (Canarini et al. 2019).

The few studies that have addressed temperature effects on tree root exudation employed either experimental soil warming of seedling cultures (Karst et al. 2017; Uselman et al. 2000; Yin et al. 2013 a, b,) or compared trees of the same species at sites differing in mean temperature (Yang et al. 2020). While most studies with trees indicate higher exudation under elevated temperatures, higher exudation at lower temperatures was also reported (Karst et al. 2017; Yang et al. 2020). Due to the multitude of possible influential factors and the difficulties associated with measuring tree root exudation under field conditions, our understanding of climate warming effects on exudation is very limited. This weakens our capacity to predict changes in soil biological activity and C and nutrient cycling in forest soils in a future warmer world.

In this study, we investigated changes in root exudation in seven mature forests of European beech (*Fagus sylvatica* L.) along an elevation transect (310 – 800 m a.s.l.) on acidic soil with the aim to explore the responses of root exudation of this tree species to both prevailing thermal conditions and varying mean annual temperature (8.4 – 6.0 °C) at the site. Earlier research in Central European beech forests with an *in-situ* cuvette-based method showed that mass-specific exudation rates decrease greatly from the topsoil to the subsoil (Tückmantel et al. 2017) and increase with soil acidity and N deficiency of the soil (Meier et al. 2020). To capture the assumed temperature effects, we here extend the sample of studied beech forests to an elevational transect on base-poor bedrock from the colline to the montane zone with a marked temperature decrease and precipitation increase, while soil pH (pH($CaCl_2$ in 0-10 cm mineral soil: 3.1 – 3.7) and soil N availability (15.2 – 19.9 g g^{-1}) varied only little. We hypothesized that the root exudation rate increases with increasing mean daily temperature due to an overall increase in the tree's metabolic activity, while the cumulative annual C flux with exudation is primarily determined by growing season length, which increases from the colder to the warmer stands.

Material and Methods

Study sites, climate, and geology

The study was conducted in seven European beech forests of mature age along an elevational gradient between 310 and 800 m a.s.l. on the eastern slopes of the Rothaar Mountains in the state of Hesse, central Germany. The study region has a cool-temperate humid climate with MAT decreasing from 8.4 to 6.0 °C and mean annual precipitation (MAP) increasing from 600 to 1200 mm yr^{-1} from the colline to the montane zone due to orographic lift of air masses that mostly arrive from western directions (German Weather Service, period 1981-2010; Table 1).The elevation transect had a length of approx. 30 km in east-west direction and ranged from the colline/submontane to the montane belt, covering a gradient in mean annual temperature (MAT) of about 2.4 °C and a MAP gradient of 600 mm yr^{-1}. The studied beech forests are thus exposed in downslope direction to increasingly warmer and drier summers, as is predicted to happen in the course of climate warming in the 21st century in Central Europe and elsewhere (Kaspar et al. 2017). By employing a space-for-time substitution, this setting may allow rough estimates of anticipated future changes in tree root exudation with climate warming. Besides temperature, we measured soil moisture and several soil chemical parameters as well as fine root biomass (FRB) and fine root morphological traits in order to relate exudation to possible controlling abiotic and biotic factors. All stands belonged to the Luzulo-Fagetum forest community (beech forests on acidic soils) and stocked on acidic bedrock (Triassic sandstone or Paleozoic clay shale) in level to slightly inclined terrain (Table 1). In the forests, study plots of 30 m x 30 m size were selected in sections with closed canopy. While mean diameter at breast height (DBH) varied only between 32 cm and 45 cm in the seven stands and tree ages ranged mostly between 100 and 180 years according to information from the forest offices, stem density was more variable (150-578 stems ha^{-1}). Mean tree height as measured in 15 trees per plot with a Vertex III height meter (Haglöf, Längsele, Sweden) with at least three measurements taken per tree from different directions decreased with elevation from 33 m to 20 m.

During the study period from spring 2014 to winter 2015, air and soil temperature were continuously measured in 2015 with iButton sensors (Maxim, Dallas, USA) installed at 1.5 m height above the forest floor and in the topsoil (3 cm depth) in the seven stands. The sensors were read every 60 min. Dendrometer tapes (type D1, UMS, Munich, Germany; precision of 0.1 mm) were permanently installed at 1.5 m height on 15 trees per plot to determine DBH and annual stem diameter increment through annual DBH recording.

Table 1. Location and physiographic characteristics of the seven study sites along the elevational gradient in central Germany.

Elevation	m a.s.l.	310	380	490	560	600	690	800
Longitude	[E]	08° 55'	08° 56'	08° 48'	08° 45'	08° 42'	08° 37’	08° 33’
Latitude	[N]	51° 19'	51° 18'	51° 17'	51° 18'	51° 17'	51° 16’	51° 17’
Inclination/ Exposition		10° SE-NW	5° NE-E	10° NW-SE	15° NW-NE	20° NE-E	20° N-E	5° SE
Vegetation type		LF	LF	mon. LF	mon. LF	mon. LF	mon. LF	mon. LF
Tree age	[yr]	129-149	81-107	146-156	158-180	98-133	169-189	162-192
Stem density	(n ha^{-1})	311	578	267	489	267	150	250
Mean tree height	(m)	32.7	23.5	13.2	24.0	24.9	21.8	20.0
Mean dbh	(cm)	44.8	31.5	32.6	34.9	35.4	44.6	41.6
Growing season length	days ≥ 10°C	171	170	159	142	151	130	125
MAT	[°C]	8.1	8.4	7.9	7.4	7.7	6.6	6.0
Mean temperature 2014	[°C]	9.6	9.9	9.4	8.9	9.1	8.1	7.5
Mean temperature. 2015	[°C]	9.1	9.3	8.9	8.3	8.7	7.6	6.9
MST	[°C]	14.6	14.5	14.1	14.8	14.2	13.8	13.3
MAP	[mm]	605	643	691	887	951	1155	1209
Annual precipitation 2014	[mm]	706	707	764	845	839	1062	1115
Annual precipitation. 2015	[mm]	571	561	621	673	696	974	1054

Vegetation type: LF - Luzulo-Fagetum; mon. LF - montane Luzulo-Fagetum. Tree age was retrieved from forest inventory data (Waldeckische Domanialverwaltung). Stem density, mean tree height, and mean diameter at breast height (dbh) were determined in August 2014. All climate data were derived from DWD (Deutscher Wetterdienst, German Weather Service, Offenbach, Germany), interpolated between neighboring measuring stations, and corrected for altitude. Climate data refer to multi-annual means (1981-2010) or annual means (temperature) or sums (precipitation) of the study year (for 2014 and 2015). MAT – mean annual temperature; MST – mean annual summer temperatures (May-Sept.) in the 1981-2010 period; MAP – mean annual precipitation in the 1981-2010 period.

Soil chemical and physical analyses

To characterize soil chemical factors, each five samples were collected in summer 2015 from the uppermost 15 cm of the soil with a 6.6 cm-diameter corer at random position in the study plots. Subsequently, the thickness of the organic layer was measured in the cored hole. All soil samples were separated into organic layer and mineral topsoil material (0-10 cm), transferred to the laboratory in a cooling box, sieved (<5 mm for organic layer material; < 2mm for

mineral soil), and stored in polyethylene bags at 4°C for further processing. Subsamples were analyzed in field-moist condition for pH (measured in H_2O: 10 g fresh soil in 25 ml deionized water, or in $CaCl_2$: 10 g soil suspended in 0.01 M $CaCl_2$) after 1 h of equilibration. Additional subsamples were dried (60°C, 48 h), ground, and analyzed for total carbon and nitrogen concentrations through gas chromatography with an elemental analyzer (vario EL III, Elementar Analysensysteme GmbH, Hanau, Germany). Since all soils were highly acidic, total C content was assumed to be organic C (SOC). The total P content was determined by ICP-OES analysis (Perkin Elmer Optima 5300 DV) after acid-pressure digestion (65% HNO_3 at 195°C for 6 h) of the ground soil samples. Plant-available phosphorus was estimated with the resin bag method according to Bowman and Cole (1987) using Dowex 1 x 8-50 anion exchange gel (Dow Water& Process Solutions, USA) that was placed for 16 h in a solution of 1 g of soil material suspended in 30 ml water (Sibbesen 1977). Extracted P was re-exchanged with NaCl and NaOH solutions and the P concentration measured in a spectrophotometer (Libra S 21, Biochrom, UK) at 712 nm after adding 5 mM hexaammonium-heptamolybdate solution (Murphy and Riley 1962).

The water content of the topsoil was determined gravimetrically in each five soil samples collected at random position in the plots every month from March to December 2015. The sampling in August and September was conducted synchronously with the collection of root exudates.

Root exudate collection

Root exudates were collected in three sampling campaigns in July of 2014, August 2015, and September 2015 in nine soil pits excavated at each site in at least 3 m distance to the nearest mature beech tree, employing the cuvette-based *in situ*-collection approach (after Phillips et al. 2008; Freschet et al. 2021). Beech fine root strands were carefully extracted from the uppermost 10 cm of the soil profile and cleaned with fine forceps and deionized water. Since organic layer thickness increases with elevation (Table 2), the sampled roots were at the uppermost sites 6 and 7 entirely located in the thick organic OF and OH layers, while a greater part of the studied root strands grew in the uppermost humus-rich mineral soil Ah-horizon in the lower plots (1-5) with somewhat thinner organic layers. After cleansing, roots were placed overnight in moist, sandy soil to allow recovery from the excavation process. On the next day, the living terminal root systems (average cumulative length of all parts of the strand c. 16.5 cm and mean diameter c. 0.45 mm) were placed into root cuvettes filled with sterile 2 mm-diameter glass beads to simulate the porosity of the soil and mechanical impedance in a

matrix free of carbon. The beads covering the root were moistened with a sterile carbon-free dilute 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 $CaCl_2$) used as a culture medium.

Table 2. Soil physical and chemical characteristics of the organic layer and the mineral topsoil (0-10 cm) of the seven beech forests along the elevational gradient (means and SE in brackets). Results for soil water content (SWC) are the means of gravimetric determination on nine sampling dates with n=5 samples per site. Different upper-case and lower-case letters indicate significant differences between sites at $p < 0.05$ (p-values adjusted by the Benjamini-Hochberg procedure for multiple comparisons).

Elevation	m a.s.l.	310	380	490	560	600	690	800
Parent material		Sandstone	Sandstone	Clay shale	Clay shale	Clay shale	Clay shale	Clay shale
Geological epoch		l BU	l BU	l K	u D	u D	m D	m D
Org. layer thickness	cm	4.5 ± 0.26	5.2 ± 0.35	7.7 ± 0.42	7.8 ± 0.39	7.9 ± 0.66	10.9 ± 0.65	10.5 ± 0.49
Org. layer mass	[kg m^{-2}]	7.97 ± 1.05	7.56 ± 1.18	8.41 ± 0.86	11.05 ± 1.01	11.21 ± 1.76	13.90 ± 1.38	15.03 ± 2.44
Texture								
0-10 cm		Sandy loam	Sandy loam	Sandy loam	Loamy silt	Loamy silt	Silt loam	Sandy Loam
Bulk density								
Org. layer	[g cm^{-3}]	0.18 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.15 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.14 ± 0.02
0-10 cm	[g cm^{-3}]	1.32 ± 0.02	1.36 ± 0.04	1.11 ± 0.03	1.07 ± 0.07	0.99 ± 0.11	0.75 ± 0.06	0.76 ± 0.04
SWC								
Org. layer	[wt %]	46.9 ± 2.1	41.4 ± 1.9	59.1 ± 1.6	57.8 ± 1.6	52.4 ± 1.7	61.3 ± 1.2	66.9 ± 1.1
0-10 cm	[wt %]	22.8 ± 0.9	18.7 ± 0.8	26.1 ± 1.3	27.1 ± 1.3	29.4 ± 1.1	38.1 ± 0.7	43.6 ± 1.4
pH (H_2O)								
Org. layer		4.3^{A} ± 0.03	4.7^{A} ± 0.38	4.2^{AB} ± 0.02	3.9^{B} ± 0.07	4.2^{AB} ± 0.03	4.2^{AB} ± 0.07	4.1^{AB} ± 0.04
0-10 cm		3.7^{b} ± 0.02	4.6^{a} ± 0.09	3.8^{a} ± 0.06	3.8^{a} ± 0.12	3.7^{b} ± 0.09	3.7^{b} ± 0.03	3.7^{b} ± 0.18
pH ($CaCl_2$)								
Org. layer		3.7^{A} ± 0.05	4.3^{A} ± 0.34	3.6^{A} ± 0.05	3.3^{B} ± 0.09	3.4^{A} ± 0.06	3.3^{B} ± 0.04	3.3^{B} ± 0.13
0-10 cm		3.4^{ab} ± 0.04	3.5^{ab} ± 0.05	3.5^{a} ± 0.03	3.3^{ab} ± 0.05	3.4^{ab} ± 0.03	3.3^{ab} ± 0.02	3.1^{b} ± 0.18
C_{tot}								
Org. layer	[mg g^{-1}]	143.7^{D}(±9.6)	175.7^{CD} ± 8.4	255.4^{AB} ± 11.6	253.6^{AB} 12.1	215.0^{BC} ± 10.8	276.5^{A} ± 14.2	304.4^{A} ± 10.5
0-10 cm	[mg g^{-1}]	26.1^{c}(±1.7)	28.1^{c} ± 1.4	52.9^{b} ± 7.2	63.3^{b} ± 7.8	56.9^{b} ± 3.6	82.4^{a} ± 3.3	111.8^{a} ± 9.4
N_{tot}								
Org. layer	[mg g^{-1}]	7.8^{D}(±0.46)	8.8^{D} ± 0.36	14.1^{BC} ± 0.56	13.1^{BC} ± 0.61	11.9^{C} ± 0.51	15.4^{AB} ± 0.7	16.7^{A} ± 0.57
0-10 cm	[mg g^{-1}]	1.6^{c}(±0.12)	1.4^{c} ± 0.09	3.2^{b} ± 0.36	3.1^{b} ± 0.34	3.5^{b} ± 0.18	4.7^{a} ± 0.17	6.1^{a} ± 0.48
C/N								
Org. layer	[g g^{-1}]	18.6^{AB} ± 0.37	19.8^{A} ± 0.42	17.9^{BC} ± 0.21	19.3^{A} ± 0.22	17.7^{BC} ± 0.28	17.9^{BC} ± 0.23	18.3^{ABC} ± 0.14
0-10 cm	[g g^{-1}]	15.2^{d} ± 0.51	19.8^{ab} ± 0.75	16.2^{cd} ± 0.51	19.9^{a} ± 0.40	16.3^{cd} ± 0.30	17.5^{bc} ± 0.16	18.3^{ab} ± 0.23
P_{tot}								
Org. layer	[mg g^{-1}]	0.82 ±0.04	0.96 ± 0.04	0.92 ± 0.04	0.92 ± 0.04	0.87 ± 0.04	0.90 ± 0.04	0.83 ± 0.05
P_{resin}								
0-10 cm	[mg g^{-1}]	0.04^{a}± 0.003	0.01^{b} ±0.003	0.01^{b} ± 0.003	0.01^{b} ±0.002	0.02^{b} ±0.002	0.01^{b} ±0.001	0.03^{a} ±0.003

Information on soil texture was provided by the local forestry offices (Waldeckische Domanialverwaltung). Geological epochs: l BU – lower Bunter (Triassic), l K – lower Keuper (Triassic), u D or m D - upper/middle Devonian shale.

P_{resin} – resin-exchangeable P.

The roots in the cuvettes were allowed to recover for 48 h before flushing and cleaning the cuvettes 3 to 5 times with culture medium using gentle, low-pressure vacuum (≤-0.2 bar) induced by a syringe. New sterile culture medium (*c.* 40 mL) was added and after a full diurnal (photosynthetic) cycle of approx. 24 h, these trap solutions containing exudates were collected for analysis. These solutions were subsequently filtered through sterile glass fiber filters (GE Healthcare Life Sciences Whatman, Glass Microfibre Filters, Grade GF/F) and stored at -20°C until further analysis. Control samples were taken from rootless cuvettes treated similarly. The samples were analyzed for their dissolved organic carbon using a total carbon analyzer (Shimadzu TOC-L CPH/CPN; Shimadzu Scientific Instruments, Duisburg, Germany). Taking fresh root biomass as a calculation basis, net mass-specific exudation rates (μg C g^{-1} h^{-1}) and annual C fluxes with exudation per root mass or ground area (mg g^{-1} yr^{-1}, g C m^{-2} g^{-1}) were calculated, the latter from site-specific exudation-temperature relationships and temperature variation across the growing season, taking all days with average temperatures >10 °C into account. This is a rough estimate, as it assumes a constant temperature dependence of exudation across the growing season. To estimate growing season length for the seven sites, we used gridded temperature data provided by the German Weather Service (DWD). Growing season length decreased with decreasing MAT from 170 to 125 d between 310 and 800 m a.s.l. (Table 1).

Fine root biomass and root morphology

In November 2018, 12 root samples were taken at random locations in each of the 30 m x 30 m plots using a soil corer (6.6 cm in diameter) and the material separated into organic layer and mineral topsoil (0-10 cm) material. Samples were transported in a cooling box to the laboratory where they were kept at 4 °C and processed within four weeks. Only fine roots (diameter < 2 mm) of beech were considered in the analysis. All fine root segments were picked out by hand and sorted into live and dead fine root mass under a stereomicroscope (40x magnification). Root vitality was assessed by means of root 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). Standing FRB was expressed as profile total (organic layer and uppermost 10 cm of mineral soil; in g m^{-2}). Specific root length (SRL, m g^{-1}), specific root surface area (SRA, cm^2 g^{-1}), root tissue density (TD, mg cm^{-3}), and root tip frequency (RTF, number of root tips per fine root mass; n g^{-1}) were determined for the root material using a flatbed scanner and the software WinRhizo

(Régents Instruments, Quebec, Canada). Fine roots used for exudate collection were clipped off and the biomass of that root segment determined by drying (48h, 78°C) and weighing the sample.

Statistical analyses

All statistical analyses were conducted with SPSS software. The data was tested for fit to normal distribution using a Shapiro-Wilk test. Normally distributed data were tested for homogeneity of variances with a Levene test. Site differences between means of edaphic (total C and N content, C/N-ratio, pH (H_2O), pH ($CaCl_2$), total and plant-available P content) and root morphological variables (TD, SRA, SRL, and RTF), and exudation rates (net mass-specific exudation rate and annual C flux with exudation) were examined with one-way analysis of variance for parametric data and a Kruskal-Wallis test for non-parametric data. ANOVAs were followed by a Scheffé or a Dunnett T3 test, if homogeneity of variances was not fulfilled. Kruskal-Wallis tests were followed by pairwise comparisons to locate differences. Pearson correlations were used for investigating the relation between root exudation rate and elevation, climatic and edaphic variables, and root morphological traits. If data were non-normally distributed, Spearman rank correlation analysis was employed. Correlations were tested for the variables long-term mean temperature and precipitation, average summer temperatures of 2014 and 2015, soil water content at the date of exudate sampling, and air and soil temperatures averaged over the seven days prior to sampling. The p-values were adjusted by the Benjamini-Hochberg procedure for multiple testing.

Multiple regression analyses with backward variable elimination were conducted to test for significant independent predictors of root exudation rates and the estimated annual C flux with exudation per m^2 ground area. As variation in soil chemical factors across sites was moderate to low due to the sampling design, and root morphology varied little, we ran the initial model with the abiotic factors site-specific daily temperature, soil moisture and elevation, and diameter at breast height and stem density as key characteristics of stand structure. At each elimination step, the variable showing the smallest contribution to the model was deleted until all variables remaining in the model produced significant *F* statistics. The p-values were calculated via the bootstrapping method because most of the data showed no fit to normal distribution. Variables were tested for multi-collinearity and were excluded when they were highly correlated and collinearity diagnostics (variance inflation factor and tolerance) were critical.

Results

Elevational change in climatic and edaphic conditions

During the two years of measurement, annual mean air temperature (weather station data) decreased at a lapse rate of c. 0.57 °C per 100 m from 9.9 to 7.5 °C in 2014 (and from 9.3 to 6.9 °C in 2015) along the elevational gradient. Both years were on average by about 1.5 °C (2014) and 1.0 °C (2015) warmer than the long-term average of the 1981-2010 period (Tab. 1). Annual precipitation increased from 706 mm at 310 m to 1115 mm at 800 m in 2014, and from 571 to 1054 mm in 2015, corresponding to average increases by 83 and 99 mm per 100-m increase in elevation during the two study years.

The recording of climatic conditions in the stands directly before exudate sampling in summer 2015 gave smaller air temperature (T_a) and soil temperature (T_s) decreases with elevation during the sampling campaigns (T_a decrease by 0.37 and 0.53 °C 100 m^{-1} and T_s decrease by 0.41 and 0.45 °C 100 m^{-1} in August and September 2015, respectively; Fig. 1a). The amount of precipitation recorded one week prior to each sampling campaign showed a very slight decrease with elevation in 2014, with the lowest amount measured at the highest sites at 800 m (244 mm) and 690 m (260 mm) and the highest amount at the intermediate sites at 490 m (291 mm) and 560 m (281 mm). Precipitation increased with elevation during the August and September 2015 sampling campaigns (Fig. 1b). Gravimetric soil water content (SWC) of the organic layer plus uppermost mineral soil (0-10 cm) determined for the sampling in August and September 2015 roughly doubled between the lowest and highest sites from 15-25 vol. % at the low-elevation sites to 38-47 vol. % at the high-elevation sites (Fig. 1c). In summer 2015, SWC increased along the gradient from 46.9 % at 310 m to 67.0 % at 800 m in the organic layer, and from 22.8 % to 43.6 % in the mineral topsoil (Table 2).

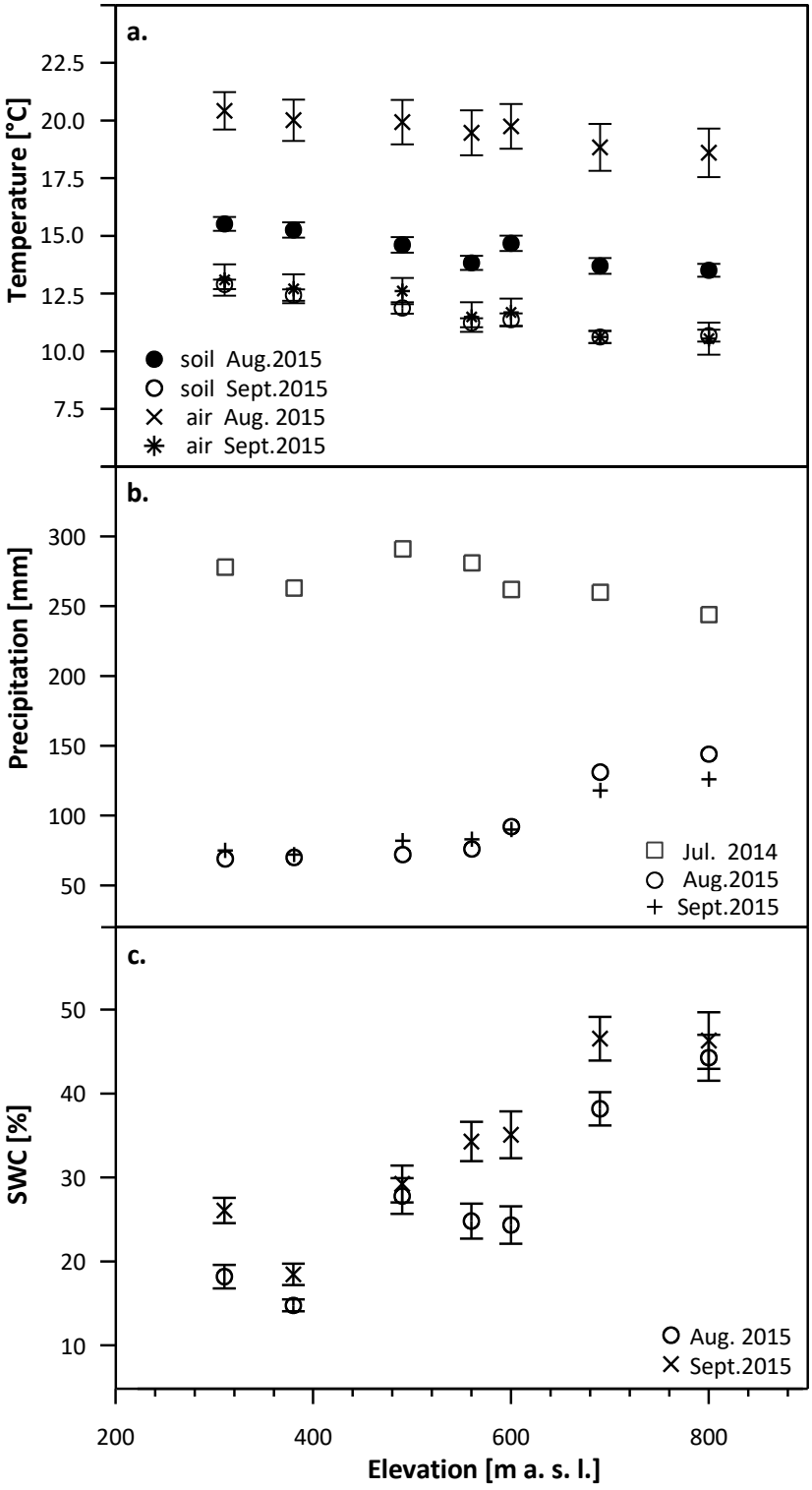


Fig. 1 Soil and air temperature (a), precipitation (b) and soil water content (SWC; wt.%) (c) measured in seven forests at two sampling dates in summer/autumn 2015 and one sampling date in summer 2014 (precipitation only) (means ± SE). Temperatures are means of seven-day periods prior to exudate sampling of each 5 sensors per stand, precipitation data are interpolated from gridded weather station data of the German Weather Service (DWD) corrected for elevation, and SWC data are gravimetric samples taken in the organic layer and 0-10 cm mineral soil at the date of sampling (n = 5).

Among the most conspicuous changes observed in soil chemical and physical factors was the doubling in organic layer thickness from about 5.5 cm at 310 m to 10.5 cm at 800 m a.s.l., which was associated with an increase in organic layer mass from 8.0 kg m^{-2} at 310 m to 15.0 kg m^{-2} at 800 m (Tab. 2) and concomitant increases in organic layer C and N stocks. The organic carbon concentration (SOC) in the organic layer material increased from 143.7 mg g^{-1} at 310 m to 304.4 mg g^{-1} at 800 m, and that of the mineral topsoil from 26 mg g^{-1} at 310 m to 111.83 mg g^{-1} at 800 m. In parallel, total N concentration increased in the organic layer from 7.8 mg g^{-1} at 310 m to 16.7 mg g^{-1} at 800 m, and in the mineral topsoil from 1.64 mg g^{-1} at 310 m to 6.05 mg g^{-1} at 800 m. Soil C/N ratio and P content showed no elevational trends in both layers (Tab. 2).

Elevational change in fine root biomass and root morphology

FRB in the organic layer increased with elevation largely in parallel with the increasing forest floor depth from 2.2 g m^{-2} at 310 m to 24.0 g m^{-2} at 690 m (significant relation; $r=0.51$, $p<0.01$), revealing a positive relation to MAP and a negative one to MAT (Table 3). FRB in the mineral topsoil (0-10 cm) varied between 9.3 and 48.9 g m^{-2} without a clear elevational trend (Fig. 2). Both FRB components were negatively related to the P concentrations in the organic layer (P_{tot}) and mineral soil (P_{resin}) but were unrelated to soil C/N ratio. None of the examined root morphological parameters (SRL, SRA, TD, RTF) changed significantly with elevation, nor were influenced by climatic or soil chemical variables (Table 3).

Elevational change in exudation rates and dependence of exudation on climatic and stand structural properties

Average mass-specific root exudation rate across the seven sites was 18.34 ± 6 µg g^{-1} h^{-1} (Fig. 4a) with site means (averaged over all sampling dates) ranging from 12.2 µg g^{-1} h^{-1} at 690 m to 21.6 µg g^{-1} h^{-1} at 380 m a.s.l. (peak rates > 37 µg g^{-1} h^{-1}). While lowest specific exudation rates were measured at the highest sites 690 and 800 m (12.2 - 15.0 µg g^{-1} h^{-1}), variation among sites was large and peak rates were recorded at mid elevation (490 m: 22.7 µg g^{-1} h^{-1}) and not at the lowest sites (Fig. 4a). Yet, the negative relation between mean exudation rate and elevation was highly significant ($r=-0.31$, $p<0.01$; Tab. 3). C flux with exudation per ground area, calculated by multiplying specific exudation with FRB in the organic layer, was significantly higher above 400 m a.s.l. than at lower elevation (Fig. 4b).

Estimated annual cumulative C fluxes with exudation per root mass, derived from site-specific exudation-temperature relationships and recorded temperature variation across the growing season (all days with means> 10 °C), decreased significantly with elevation along the transect, from 80-85 mg g^{-1} yr^{-1}at 310 m to 40-45 mg g^{-1} yr^{-1} at 800 m a.s.l. (mean of the seven sites: 66 ±29 mg g^{-1} yr^{-1}; Fig. 4c). Estimating annual C flux per ground area by multiplying the cumulative, mass-specific C flux with the FRB total of the organic layer and mineral topsoil yields a different elevational pattern. Highest stand-level C fluxes were calculated for 380 m (ca. 4.6 g C m^{-2} yr^{-1}) and 600 m (ca. 3.2 g m^{-2} yr^{-1}) with highest FRB, and lowest for 310 m (ca. 0.8 g m^{-2} yr^{-1}) and also 490 m (ca. 1.6 g m^{-2} yr^{-1}), but without a dependence on elevation (Fig. 4d). Averaged over all sites, we calculated a mean growing season C flux of 2.2 ± 0.2 g m^{-2}.

The environmental factors with strongest influence on mass-specific root exudation rate were the site-specific daily air and soil temperatures with highly significant positive effects ($r=0.66$, $p<0.01$; Table 3a), revealing a linear increase of exudation rate at a slope of 1.97 µg g^{-1} h^{-1} per 1 °C temperature increase (Fig. 5a; Fig. S1 in the Supplement). In contrast, the relationships to MAT and MST and mean temperature of the measuring year were less tight ($r=0.29$-0.31, $p<0.01$ or n.s.). Close relations were also found for soil moisture (SWC) and MAP (both negative), and the N content of the upper soil (negative) and soil pH ($CaCl_2$) (positive), while soil P content and C/N ratio were not influential (Table 3a). In contrast to actual exudation rate, the annual stand-level C flux depended only on the P_{resin} concentration of the topsoil (negative relation; $r=0.25$; $p<0.05$) and specific root area (positive relation; $r=0.25$; $p<0.05$), but not on any climatic factor. Annual fluxes consequently were related neither to actual (average summer) temperature nor to long-term mean summer temperature (MST) (Fig. 5c and d).

While FRB decreased with increasing stem density ($r=0.36$), specific root exudation rate did increase ($r=0.32$; Table 3b and Fig. S2 in the Supplement).

Multiple regression analyses on climatic and stand structural drivers of root exudation

Multiple regression analyses on the influence of various climatic and stand structural factors on the exudation rate of beech indicate a dominant positive effect of the site-specific daily air temperature ($F=69.7$; $p<0.001$), followed by a somewhat weaker negative effect of diameter at breast height (DBH; $F=39.3$; $p<0.001$). The model explained 41 % of the variance in exudation rate (Table 4). Annual C flux per ground area was influenced only by DBH, but not by temperature or any other climatic variable; the explained variance was only 14 %.

		FRB org.lay.	FRB 0-10cm	SRL	SRA	TD	RTF	Root exudation rate	Annual C Flux
		(g m $^{-2}$)	(g m $^{-2}$)	(cm g $^{-1}$)	(cm^2 g^{-1})	(mg cm^3)	(n g^{-1})	(µg g^{-1} h^{-1})	(g m^{-2} yr^{-1})
a)									
Elevation	(m a. s. l.)	**0.510****	0.093	-0.032	0.032	-0.013	0.082	-0.305**	-0.070
MAP (1981-2010)	(mm)	**0.510****	0.093	-0.032	0.032	-0.013	0.082	-0.305**	-0.075
MAT (1981-2010)	(°C)	-0.466**	0.104	0.038	-0.044	0.009	-0.069	0.305**	0.116
MST (1981-2010)	(°C)			0.097	0.076	-0.005	0.003	0.291	0.116
Ann. precip. 2014, 2015	(mm)	-	-	-0.024	0.009	0.113	0.087	-0.288**	-0.063
Ann. temp. 2014, 2015	(°C)	-	-	0.049	-0.041	0.068	-0.039	0.313**	0.116
Mean SWC (topsoil)	(wt%)	-	-	-0.014	0.077	-0.043	0.113	-0.495**	-0.187
Actual precipitation	(mm)	-	-	-0.022	-0.016	0.182	0.051	-0.169	-0.211
Daily air temperature	(°C)	-	-	0.092	0.066	-0.116	-0.056	**0.669****	0.068
Daily soil temperature	(°C)	-	-	0.090	0.057	-0.097	-0.060	**0.656****	0.185
C_{tot}									
Org. layer	[mg g^{-1}]	0.496**	-	-	-	-	-	-	-
0-10 cm	[mg g^{-1}]	-	-0.023	-	-	-	-	-	-
Upper soil	[mg g^{-1}]	-	-	-0.042	0.038	-0.024	0.058	-0.303**	-
Upper soil	[g m $^{-2}$]	-	-	-	-	-	-	-	-0.059
N_{tot}									
Org. layer	[mg g^{-1}]	0.496**	-	-	-	-	-	-	-
0-10 cm	[mg g^{-1}]	-	-0.042	-	-	-	-	-	-
Upper soil	[mg g^{-1}]	-	-	-0.044	0.061	-0.055	0.058	-0.320**	-
Upper soil	[g m $^{-2}$]	-	-	-	-	-	-	-	-0.179
C:N									
Org. layer	[g g^{-1}]	0.037	-	-	-	-	-	-	-
0-10 cm	[g g^{-1}]	-	0.315	-	-	-	-	-	-
Upper soil	[g g^{-1}]	-	-	0.081	0.060	0.04	0.014	0.080	0.075
pH H_2O									
Org. layer		-0.373**	-	-	-	-	-	-	-
0-10 cm		-	0.103	-	-	-	-	-	-
Upper soil		-	-	0.015	-0.078	0.023	-0.068	0.198	0.116
pH ($CaCl_2$)									
Org. layer		-0.432**	-	-	-	-	-	-	-
0-10 cm		-	0.037	-	-	-	-	-	-
Upper soil		-	-	0.013	-0.052	0.006	-0.075	0.244**	0.129
P_{tot}									
Org. layer	[mg g^{-1}]	-0.345**	-	0.147	0.024	0.125	0.075	0.204	-
Org. layer	[g m $^{-2}$]	-	-	-	-	-	-	-	0.045
P_{resin}									
0-10 cm	[mg g^{-1}]	-	-0.326**	0.061	0.015	0.145	0.105	0.020	-
0-10 cm	[g m $^{-2}$]		-	-	-	-	-	-	-0.246*
b)									
stem density	(n ha^{-1})	-0.360**	0.073	0.081	0.014	0.029	-0.033	0.323**	0.135
SRL	(cm g $^{-1}$)	-0.020	0.046	-	-	-	-	-	-
SRA	(cm^2 g^{-1})	0.068	0.049	-	-	-	-	-	-
TD	(mg cm^3)	-0.079	-0.096	-	-	-	-	-	-
RTF	(n g^{-1})	0.084	0.017	-	-	-	-	-	-

Table 3. (a) Results of Spearman rank correlation analyses on the dependence of fine root biomass (FRB), root morphological parameters (specific root length - SRL, specific root surface area - SRA, root tissue density - TD and root tip frequency - RTF), mass-specific root exudation rate, and extrapolated annual C flux with exudation on elevation, mean annual precipitation (MAP), mean annual temperature (MAT), multiannual summer temperature (MST), mean soil water content (SWC) in summer, precipitation immediately

before sampling, and air and soil temperature 7 days prior to sampling, and various soil chemical parameters in the organic layer, mineral topsoil (0-10 cm) and of the pooled organic layer and mineral topsoil (n=5 measurements per layer and site, n=2 sampling dates in 2015). (b) Results of Spearman rank correlation analyses on the dependence of fine root biomass (FRB), root morphological parameters (SRL, SRA, TD and RTF), and mass-specific root exudation rate and extrapolated annual C flux with exudation on stem density, SRL, SRA, TD and RTF. Given are the Spearman correlation coefficients and the significance of the relationship (*: $p < 0.05$, **: $p < 0.01$). Positive correlations are indicated by positive r values, negative ones by negative r values. p-values were adjusted by the Benjamini-Hochberg procedure for multiple comparison. Correlation coefficients >0.5 are printed in bold.

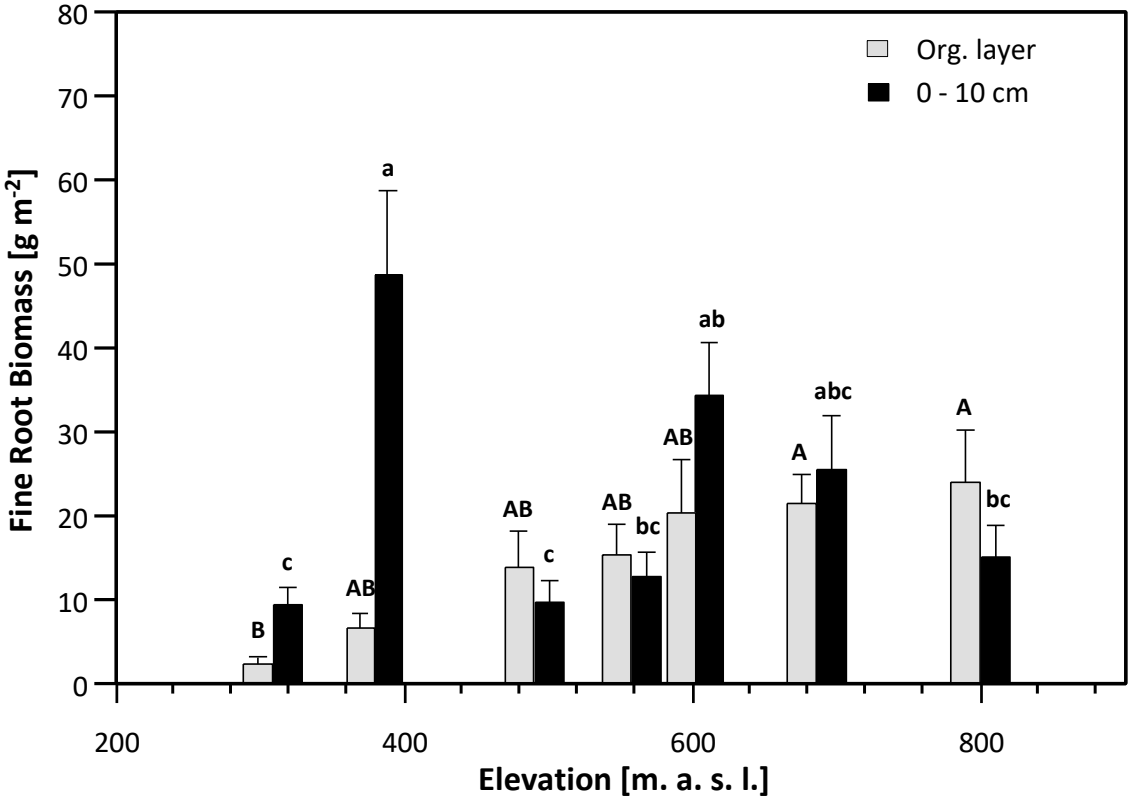


Fig. 2. Fine root biomass of beech in the organic layer (grey bars) and the mineral topsoil (0-10 cm) of the seven beech forests in November 2018 (means ± SE of 12 samples per layer). Different letters indicate significantly different means of organic layer samples (capital letters) and mineral soil samples (small letters).

Discussion

The measured mass-specific root exudation rates of the mature beech trees in our study (site means of 12-22 µg C g^{-1} h^{-1}) were similar to rates reported in other studies for beech in moist soil (10-23 µg C g^{-1} h^{-1}, Liese et al. 2017; 16-65 µg C g^{-1} h^{-1}, Meier et al. 2020) and corresponded also to values found in other tree species in the temperate zone (e.g., black locust: 10-22 µg C g^{-1} h^{-1}, Uselman et al. 2000; loblolly pine: 12-26 µg C g^{-1} h^{-1}, Meier et al. 2013). Our simple and multiple regression analyses indicate that the thermal conditions during and 7 days prior to sampling are an important factor controlling exudation of beech across the studied elevational gradient. Exudation per root mass increased by about 2 µg C g^{-1} h^{-1} per 1 °C temperature increase. In two Mediterranean tree species, Jakoby et al. (2020) found a linear increase in exudation rate per root surface area of about 0.15 µg C cm^{-2} d^{-1} per 1 °C increase, and soil temperature together with soil moisture explained exudation dynamics best in one of the species. Uselman et al. (2000) found a 70% higher root exudation in *Robinia pseudoacacia* seedlings when temperature was increased from 26 to 30 °C.

Several explanations of a positive temperature effect on exudation are possible, which relate to the 'push' and 'pull' hypotheses of the control of C acquisition by roots (Farrar et al. 2003). In the humid climate of our study region, higher summer temperatures are usually related to higher insolation and thus greater carbon assimilation, as beech photosynthesis is primarily limited by radiation at montane elevation (Schulze 1970). Consequently, under warmer conditions, more C should in principle be available for allocation to roots, which might enhance root exudation due to higher levels of non-structural carbohydrates in roots (Prescott et al. 2020) and a steeper concentration gradient of soluble organic compounds between root cells and the soil (Jones et al. 2004). The photosynthetic capacity of beech at montane elevation in Central Europe reveals a pronounced seasonality with a peak in the warmest months (Schulze 1970), which might imprint on the seasonality of exudation, as photosynthetic capacity and root exudation have been found to be closely related (Sun et al. 2017). Another possible explanation focuses on the role of root exudation for the metabolic activity of rhizosphere biota and its stimulating effect on nutrient supply for root uptake (Jones et al. 2004). Warmer weather likely increases the plant demand for nitrogen and other nutrients as photosynthetic capacity is ramped up, which might trigger roots to stimulate soil microbial activity through active secretion of labile C as an energy source for microbes (Pausch and Kuzyakov 2017). Such a mechanism would fit to the 'pull' hypothesis, as exudation then were primarily controlled by factors other than C supply from the canopy (Karst et al. 2017).

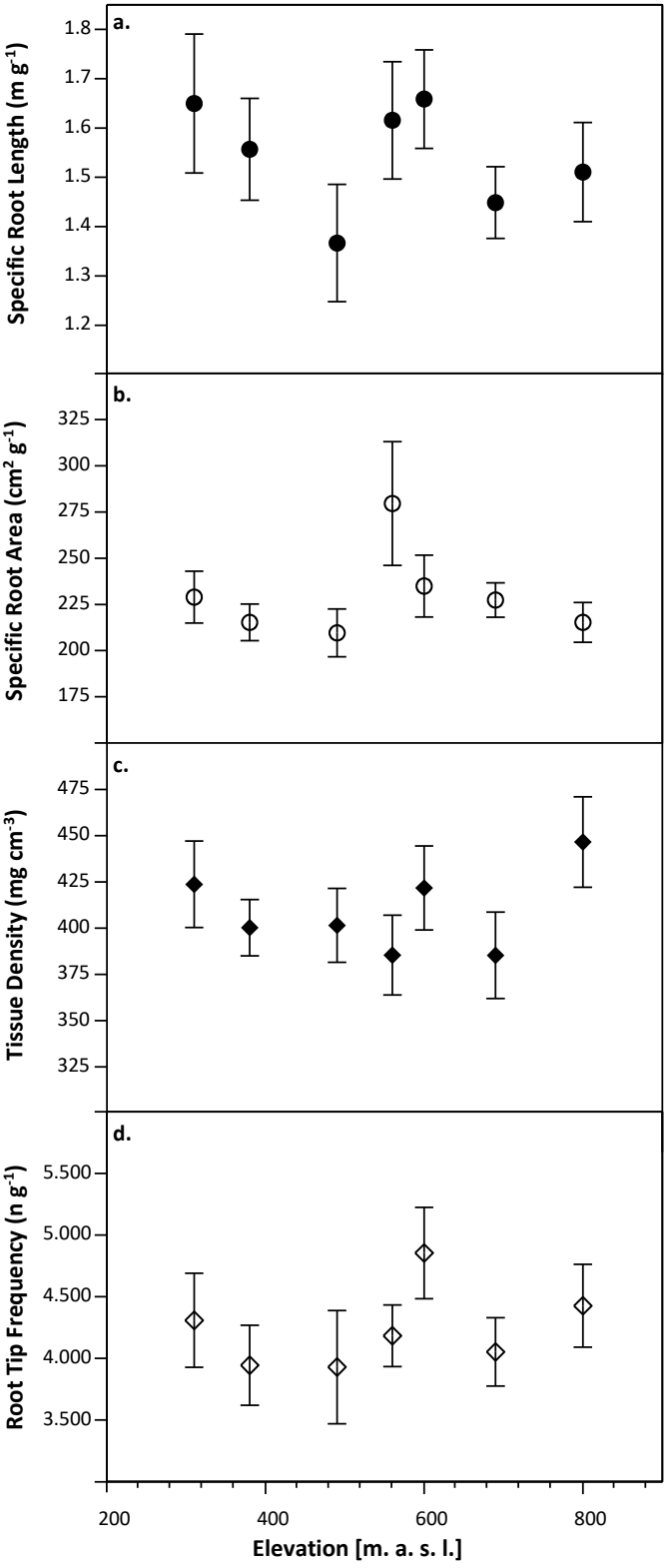


Fig.3. Specific root length, specific root area, root tissue density and root tip frequency of the fine root biomass samples used for exudation collection in the seven forests. Shown are means and standard errors of n = 9 samples per site and sampling date with averaging over the three sampling dates.

It is possible that both ‘push’ and ‘pull’ mechanisms are underlying the observed increase in exudation with rising temperature.

The fact that exudation was responsive to increases in the actual air and soil temperature at the site but showed no significant relation to mean growing season temperature (MST), i.e. the long-term average thermal conditions of the site, may suggest thermal acclimation of exudation to the mean temperature at the sites.

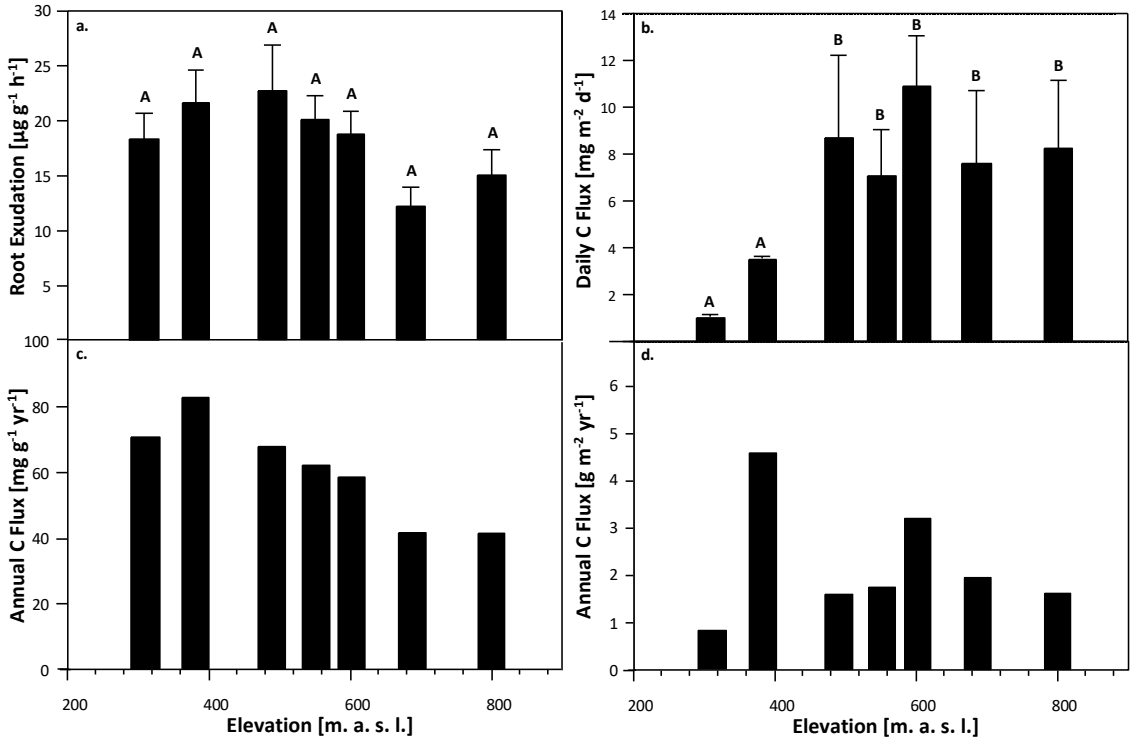


Fig. 4. Means (and SE) of (a) measured average mass-specific root exudation rate, (b) daily exudation for the fine root mass in the organic layer expressed per m^2 ground area, (c) extrapolated annual mass-specific carbon flux with exudation, and (d) estimated annual C flux with exudation per m^2 ground area in the topsoil of the seven beech forests along the elevation gradient (averaged over n = 9 samples taken per date and site and n = 3 sampling dates in 2014 and 2015). Annual exudation (c and d) was estimated from the site-specific exudation-temperature relationship, temperature variation across the vegetation period, and the specific length of the vegetation period (number of days with mean temperature ≥ 10°C) at the sites. As we calculated annual totals with pooled data, no SD can be given in figures c) and d). The exudation flux per ground area was extrapolated using the fine root biomass data from the organic layer (b) or the organic layer and the mineral topsoil (0-10 cm) (d). Different capital letters denote significantly different means at $p < 0.05$ with p-values adjusted by the Benjamini-Hochberg procedure for multiple comparisons (Kruskal-Wallis test).

Since other processes of plant carbon turnover such as respiration and photosynthesis are subject to marked thermal acclimation (Atkin and Tjoelker 2003; Hikosaka et al. 2006) and exudation depends on photosynthetic C gain, we would indeed expect that beech trees growing at lower elevation in a warmer climate down-regulate their exudation rate to a certain degree, at least that fraction of exudates that is controlled by the plant. Mean exudation rates would then become more similar across the elevational gradient. More data from field studies along temperature gradients are needed to test this hypothesis.

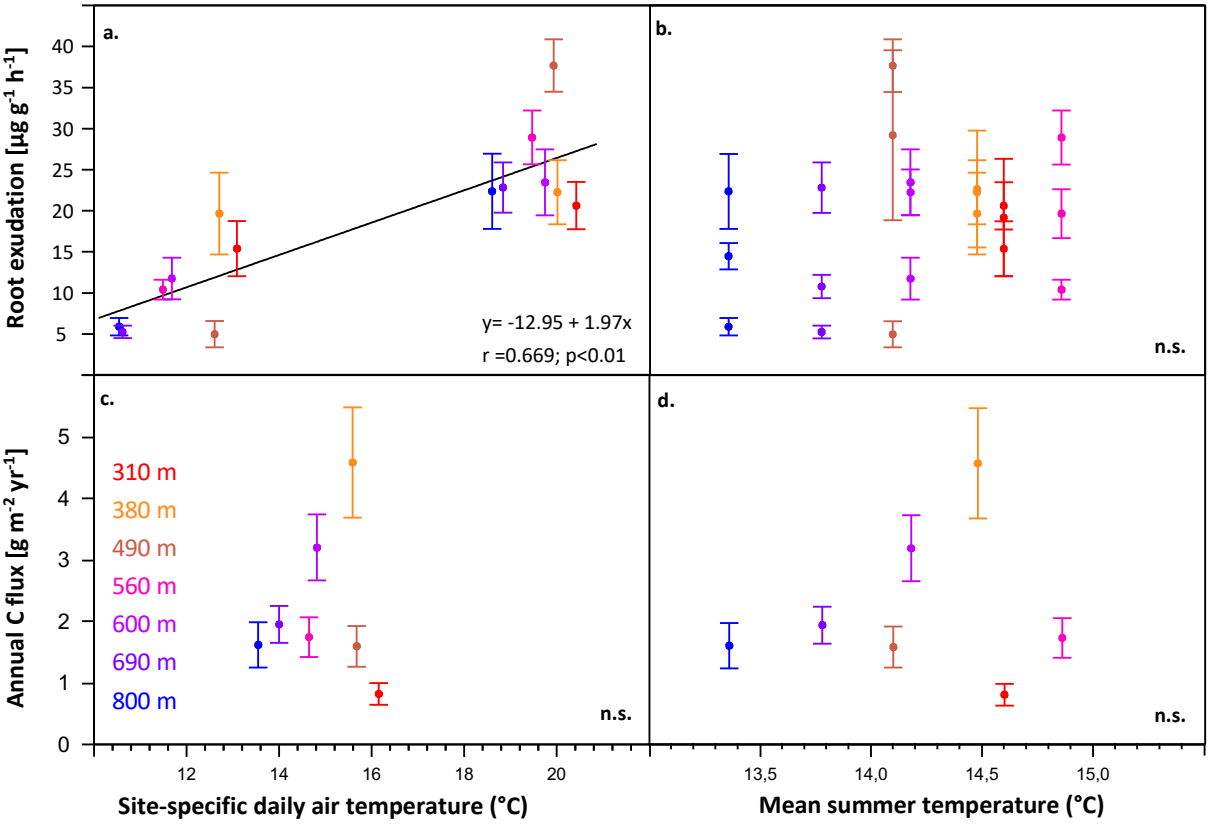


Fig. 5. Relationships between site-specific daily air temperature (means of the seven days prior to exudate measurement; left panels) or long-term mean summer air temperature (right panels) and (a) and (d) mass-specific root exudation rates, (b) and (e) cumulated annual C exudation per root mass, and (c) and (f) cumulated annual C exudation per ground area in the seven beech forests along the elevation gradient in summer 2015 (averaged over n = 9 samples per date and site; only 2015 data considered). Temperature data were measured with I-button loggers at the sampling sites.

Annual rates take the variable length of the vegetation period (no. of days ≥ 10°C) at the sites into account. Different colour of symbols indicates elevation of sites.

Since our model considers only several climatic, soil hydrologic and stand structural factors, while ignoring other likely influencing factors (such as photosynthetic activity and mycorrhizal infection) and covering other drivers only marginally due to limited parameter variation in our sample (notably soil N and P availability), we cannot assess the relative importance of temperature as a determinant of root exudation in beech. However, the positive relation of exudation to temperature is remarkably strong with a correlation coefficient of 0.66, suggesting an important role for summer temperatures and the duration of warm summer periods for exudation in beech.

Table 4. Multiple regression analyses with backward variable elimination on the effects of elevation, site-specific daily air temperature, soil water content (SWC) and elevation, and diameter at breast height and stem density on root exudation rates and the estimated annual carbon flux per m^2 ground area in the topsoil of the seven beech forests along the elevational gradient. The +/- signs indicate positive/negative correlation.

	Model					
Y	***R^2***	***P***		**Predictor**	***F***	***P***
Exudation rate	0.41	<0.001	+	Site-specific daily air temperature	69.7	<0.001
			-	DBH	39.3	<0.001
Annual C flux per ground area	0.14	0.001	-	DBH	13.1	0.001

Soil moisture (SWC) is another abiotic factor with a presumably large effect on exudation. Low to moderate drought seems to increase exudation (Jakoby et al. 2020; Liese et al. 2017; Preece et al. 2018), apparently as a stress response similar to the exudation increase observed upon soil cooling to 4 °C (Karst et al. 2017). The seven beech forests in our study represent a precipitation and soil moisture gradient with annual precipitation in the study year 2015 decreasing to nearly a half (1050 – 570 mm) and mean soil moisture in summer to less than a half (ca. 45 to ca. 20 wt.%) from 800 to 310 m elevation. In fact, the highest exudation rates were measured at the driest sites and the negative relationship to mean soil water content was highly significant, which may offer an alternative explanation of the exudation pattern along the slope, apart from a positive temperature effect. However, the correlation of exudation to soil moisture was less tight than to temperature, and SWC was not included in the multiple

regression model. Moreover, mid and late-summer moisture contents in the topsoil of 15-25 wt.% likely have exposed the trees to only mild drought, which may question that exudation was stimulated by drought in our sample. It thus remains unclear, how important water availability is for the explanation of the observed exudation patterns.

Soil nutrient availability is another abiotic factor with a possible influence on the exudation rate. For example, P deficiency results in enhanced root secretion of phenolic compounds in certain species (Neumann and Römheld,1999), and exudation of beech is higher at acidic, N-poor sites than in more fertile soils (Meier et al. 2020), which may trigger increases in decomposition rate and thus N availability through a rhizosphere priming effect (Chen et al. 2014; Jones et al. 2004; Phillips et al. 2011). The negative correlation between topsoil N content and exudation rate in our sample suggests a stimulating effect of N deficiency on exudation. There are, however, also reports of no influence of soil nitrogen deficiency on exudation, or stimulation by N addition (Uselman et al. 2000; Yin et al. 2013b). Along the studied elevational gradient, organic layer mass on the forest floor doubled from 310 to 800 m a.s.l. with the consequence that topsoil C and N pools increased, whereas C/N ratio, P_{tot} and P_{resin} content as well as soil pH did not change, suggesting that plant-availability of N and P varied only little. Gradients with larger variation in N and P availability are needed to study the relative importance of nutrient availability on the hand, and temperature on the other, on exudation in beech.

We found only minor alterations in beech fine root morphology from 310 m to 800 m a.s.l.; yet, the structure of the fine root system changed markedly. FRB in the organic layer increased roughly tenfold in parallel with the increasing depth of this layer, while FRB in the mineral topsoil varied without a clear elevational trend. We interpret this pronounced shift of FRB to the surface layer as a consequence of decreasing litter decomposition rates toward higher elevations, prompting the beech trees to concentrate their fine root mass in the organic surface layers with highest mineralization rates. This change in root distribution patterns was associated with apparent change in exudation per unit soil volume: While mass-specific exudation rate was somewhat lower, FRB was much larger in the topsoil of the high-elevation beech forests with the consequence that daily exudation rates per topsoil volume were significantly higher above 400 m elevation where the climate is cooler. Higher root densities in colder environments have been observed in northern as well as high-elevation forests (Helmisaari et al. 2007; Kubisch et al. 2016; Moser et al. 2011). They might serve two purposes, to increase the absorbing root surface area under conditions of reduced supply and to enhance the stimulation of microbial activity under low temperatures. Lower mineralization rates in cooler soil should increase the necessity for the trees to conduct rhizosphere priming

by stimulating soil microbial activity through the provision of labile C as an easily accessible energy source.

Conclusions

Our study of root exudation of mature beech trees in seven forests along an elevation (and associated temperature) gradient provides evidence that mass-specific exudation increases with temperature at the time of measurement, whereas the dependence on the site's average summer temperatures was weak. Although we fully recognize the limitations of our data set with an only short MST gradient and only three measuring campaigns at the seven sites, our results are convincing with respect to the prominent positive temperature effect on exudation, which was more important than effects of soil moisture, precipitation or stand structure. With data on FRB at the sites and information on the length of the growing season, we were able to compare the seven forests with respect to calculated exudation per ground area and to give rough estimates of cumulative exudation per growing season. Both a higher mass-specific exudation rate and a longer growing season length contribute to a generally higher cumulative exudation at the warmer sites. Future research should investigate whether higher exudation and thus more intense rhizosphere priming is a factor that contributes to the generally higher availability of N, P, and other nutrients in soil when temperature increases. Studies on temperature-dependent changes in the composition of exuded substances are another promising field of future study. In combination with earlier studies in European beech forests across edaphic and climatic gradients (Meier et al. 2020; Tückmantel et al. 2017), this study deepens our understanding of environmental controls of root exudation in this model tree species.

Acknowledgements

We thank Mechthild Stange and Irmgard Gerstmann for their skillful support in the root analyses and Hessen Forst for the permit to work on the study sites.

Supplementary Information The online version contains supplementary material available at https:// doi. org/ 10. 1007/ s11104- 022- 05629-5.

Supplement

Figure S1. Relationships between site-specific daily air (upper panel) and soil temperature (lower panel) and mass-specific root exudation rate (shown are all individual measurements at the 7 sites).

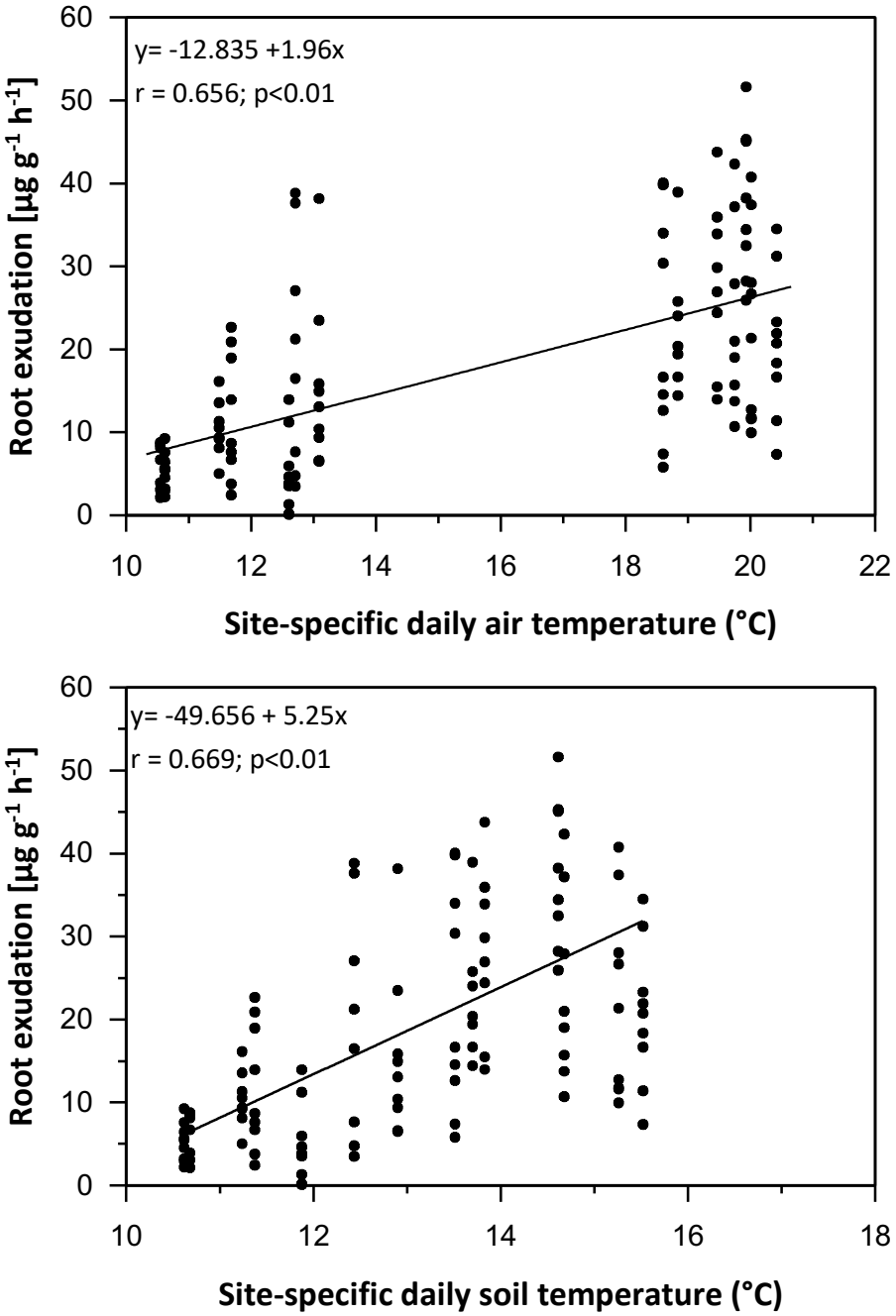


Figure S2. Mass-specific root exudation rate in relation to long-term mean annual temperature (MAT) and precipitation (MAP), and long-term mean summer temperature (MST) (period 1981-2010), annual precipitation in 2015, and tree height, stem density and diameter at breast height (DBH) in the seven stands.

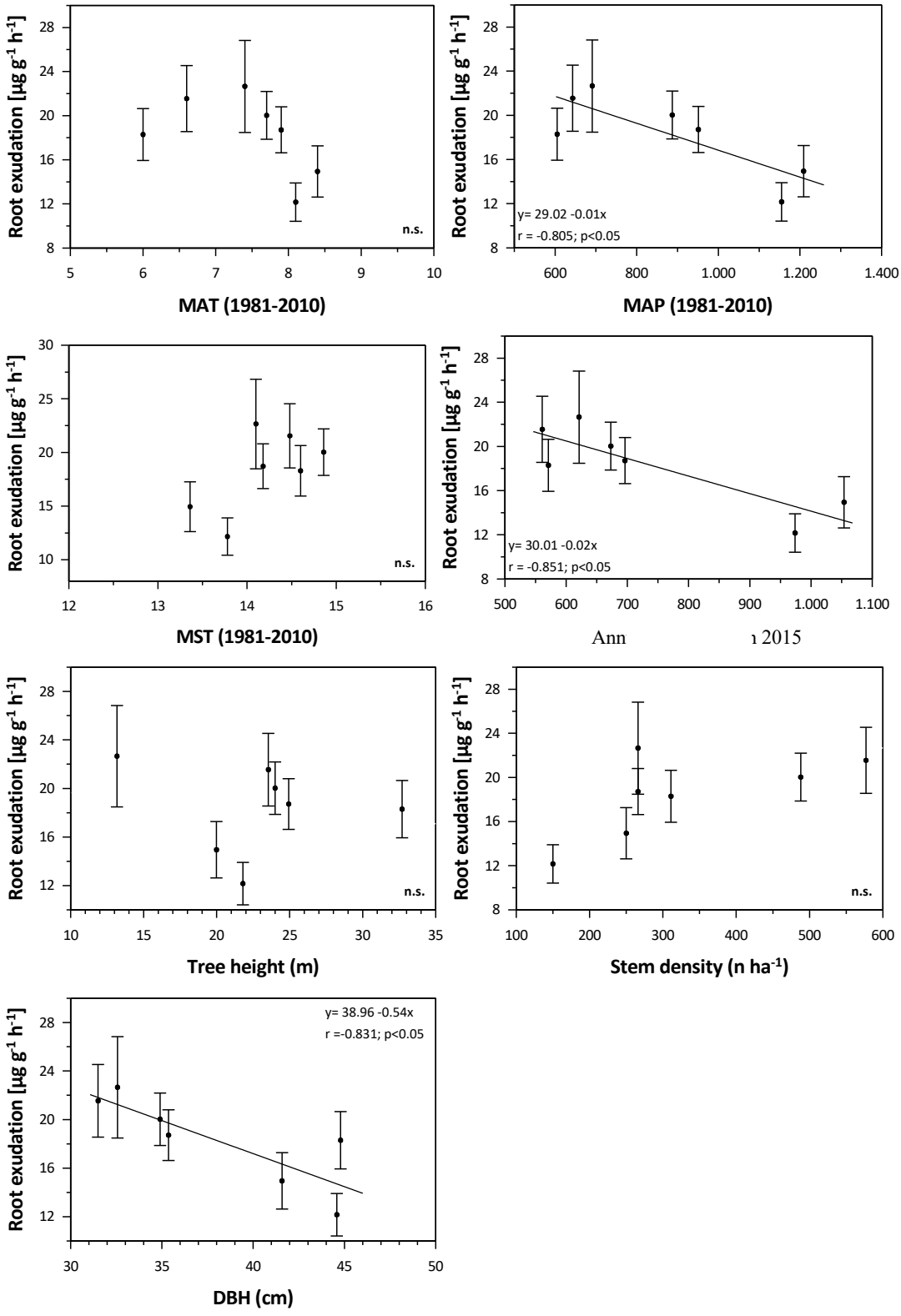
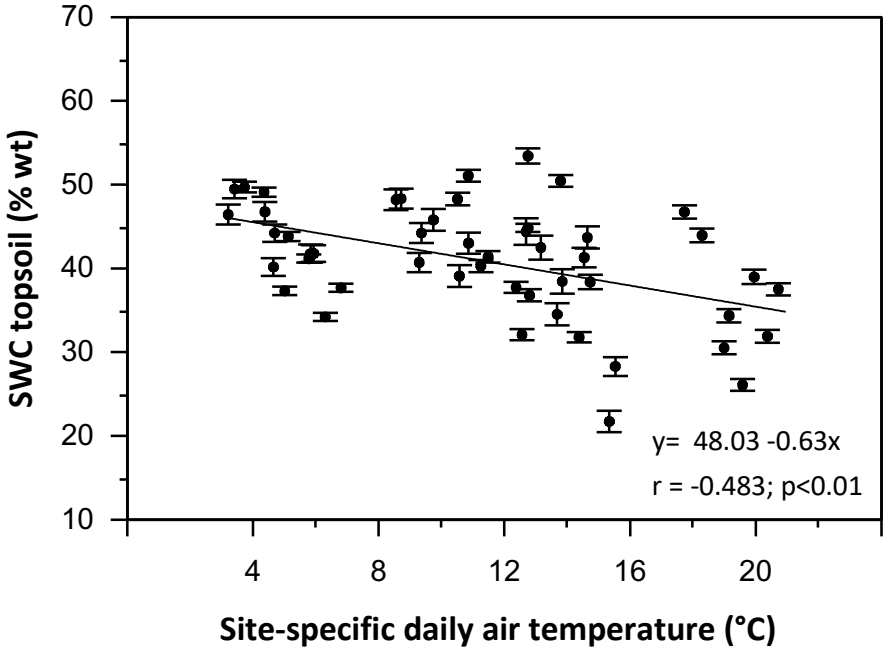


Figure S3. Relationship between soil water content (uppermost 10 cm of the profile) and daily air temperature in the sample of 7 stands.



CHAPTER 5

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SYNOPSIS

Forest soils represent important carbon (C) storages, and their relevance as a sink will increase significantly in the course of climate change induced environmental transformations in the future. The soils of beech forests are relevant for the assessment of carbon pools in Central Europe due to their wide distribution. Rhizodeposition as a key input factor of C into forest subsoils is of central importance; in particular, root exudation, which influences a wide variety of processes, is in the centre of interest. The information available to date from experiments conducted *in situ*, is fragmentary; the majority of information deals with the effects of root-borne carbon in the soil resp. rhizosphere -- the importance of ecological factors impacting these carbon fluxes are scarcely explored. The data obtained in this study will help to characterize environmental influences on a somewhat broader basis. The focus has been set on different groups of influencing factors in edaphy (depth and nutrient availability) and climate, using gradual approaches, and how they quantitatively influence the proportion of rhizodeposition that exudation accounts for.

Three projects highlight different groups of factors

Root-borne carbon fluxes appear to be regulated by a variety of internal and external factors. Internal factors describe, for example, processes of distribution of assimilates within the plant according to local demand with regulation of the adjustment of C release via the root to compensate for gaps in nutrient supply by means of resource acquisition within the rhizosphere (Prescott et al., 2020) . External factors are, for instance, the complex interconnected soil properties such as the presence or absence or spatial distribution of degradable organic soil material, the soil chemistry and the composition and abundance of the microbial fauna and the proportion of mycorrhizal fungi, but also climatic factors such as temperatures and precipitation, which affect assimilate fixation (Chen et al., 2014; Wang et al., 2016; Williams & de Vries, 2020a). As the uptake of photosynthetically bound C into the root for exudation is significant (up to one third, Liese et al. 2018), the impact of environmental controls on photosynthetic gain is correspondingly meaningful.
The three projects in this study were used as an approach to highlight individual, variable factor complexes under relatively comparable conditions, respectively to assess their impact on carbon release by the root. The connection is, that the estimated exudation fluxes can be compared with each other and provide a rough estimate of the magnitudes of influence that different parameter groups of soil depth, soil chemistry, nutrient supply and climate have.

Figure 1 shows in simplified form the processes that induce nutrient release and ultimately plant benefit through microbial priming and subsequent decomposition of SOM. Under A, B and C the investigated factor complexes (projects) of this study are shown, a variation of these groups changes the factors indicated by arrows.

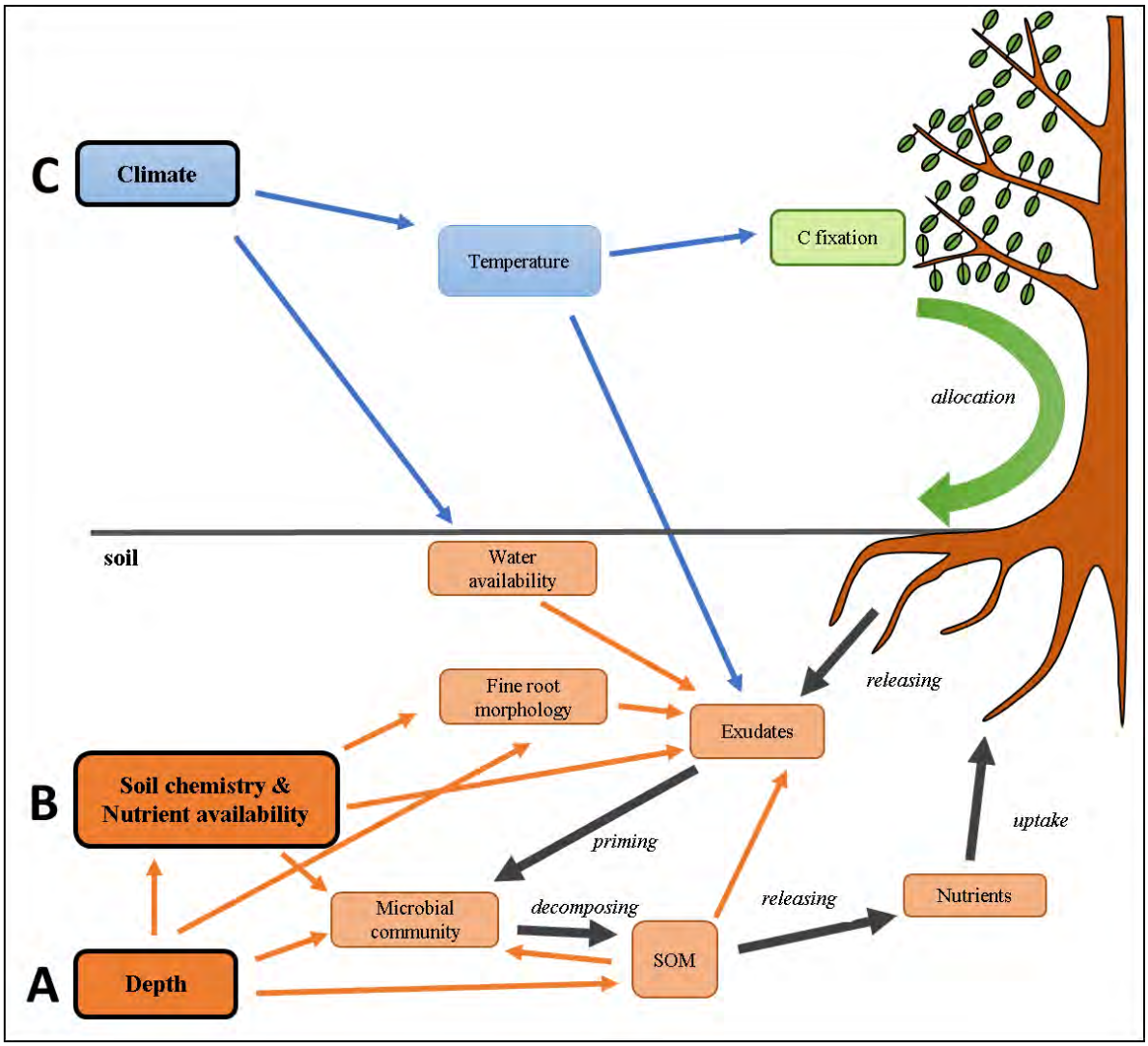

Figure 1 Simplified illustration of the processes and factors that influence root-borne carbon release or frame its magnitude. Highlighted here is only the component that promotes microbial priming. Black arrows show the pathway or use of exuded carbon with subsequent benefits for the plant, light brown arrows show relationships between soil factors, light blue arrows climatic relationships. The highlighted groups (A-C) show the factor clusters that were gradually investigated in this study.

The adapted formation of fine root morphology in soil areas or under soil conditions where priming appears to be of little use gives the impression of a plastic control mechanism that morphologically throttles rhizodeposition as spatially required. Higher demand, e.g. due to the presence of degradable organic carbon compounds, requires sufficiently large metabolically

active surfaces that provide the capacity for sufficient C-release. The demand is also regulated by various soil properties, such as soil acidity and the resulting change in the proportion of fungal biomass. The need for deposited compounds that have an impact in the soil seems to be regulated by given conditions or resources through corresponding formation of morphology.
Climatic factors such as temperature and solar radiation intensity or precipitation and soil moisture affect photosynthetic capacity, which is reflected in the supply of assimilates. Thus, appears to be a variable provisioning capacity or throttling.

Studied gradient	Soil depth	Nutrient availability	Elevation transect	
Exudation rate	5.0 - 82.0	18 - 65	12.2 - 21.6	µg C g^{-1} h^{-1}
C flux	1.0 - 52.0	4.5 -15.5	2.0 - 6.0	g C m^{-2} yr^{-1}
Main drivers	TD SRA SON SOC	SRL pH enzyme activity	soil water content air temperature	

Table 1 Overview of the exudation rates and C-flows determined in the different projects. Among them, the factors of root morphology (TD: tissue density, SRA: specific root area, SRL: specific root length), availability of mineral associated SOC and SON in the soil (SOC: soil organic carbon, SON: soil organic nitrogen), extracellular enzyme activity as well as soil water content and air temperature are shown, which were related to root derived C release.

Table 1 shows comparatively the fluctuations of the measured exudate rates and carbon fluxes in the soil. The fluctuations that were related to soil factors, like nutrients or chemistry, are much higher, up to 4-fold in maximum, than those that could be interpreted as temperature- and SWC-related. In the soil, depth-related changes in morphology, which correspond to decreases in org. N and min. assoc. SOC, appear to have the strongest impact, compared to changes that were interpreted by altered soil chemistry.

Root exudation, nutrient availability and influences on carbon pools

Several studies indicated that there are relationships between the presence and availability of nutrients in the soil and the spatial accumulation and morphology of fine roots and further the amount of exudates released (de Vries et al., 2016; Neumann & Römheld, 1999; Phillips et al., 2011). In ectomycorrhizal (ECM) dominated forests, which include beech stands, most soil nutrients are present in organic compounds; mining of organic soil stocks is dependent on

microbial decomposition and, to a considerable extent, priming effects (Phillips et al., 2013; Yin et al., 2014). Therefore, the magnitude at which labile carbon is exuded is related to the abundance of exploitable soil organic material (SOM) and, to a considerable extend, N (Chen et al., 2014) and the environmental conditions that determine microbial priming. In this context, the `microbial mining´ theory assumes the use of labile C as an energy source to decompose recalcitrant SOM for mobilizing N by microbes with N-demand (Blagodatskaya & Kuzyakov, 2008; Wang et al., 2015).

In this study, strong decreases of N, P and extractable organic carbon (EOC) with increasing depth were observed in the subsoil of glacial deposits (Chapter 2). This was accompanied by strong decreases in exudation rates and microbial biomass. C : N ratios based on soil organic matter were calculated based on soil samples from the different experimental plots along the nutrient availability gradient (Chapter 3) and showed a significant increase at the three locations characterized by acidic soils. This was significantly related to annual C-fluxes from exudation; indicating increased exudation, when organic nitrogen contents were smaller. This highlights the importance of SOM composition for priming effects with respect to the theory of `microbial mining´, since the amount of labile C excreted is linked to specific proportions of organic C and N, as well as microbial abundance in the soil to ensure optimal utilization of the released C for the purpose of efficient N mobilization. In this context, it should be noted, that the proportion of mineral N is highly dependent on soil conditions. The availability of mineral N depends on the ratio of ammonium to nitrate in the soil. Ammonium is oxidized to nitrate when soil conditions permit nitrification. The activity of nitrifying microorganisms is inhibited under anaerobic conditions, both low and high temperatures, as well as acidic soil conditions (Miller & Cramer, 2005); resulting in ammonium dominance in soils that are wet, cold, and characterized by low pH. This is promoted by the different diffusivity of nitrate and ammonium in soil. Ammonium, due to its positive partial charge, is generally less available in the dissociated state because it binds to soil minerals (i.e., strong binding to clay), while nitrate is more mobile. Excess nitrate is leached easily into deeper soil layers or into the ground water if soil moisture conditions allow percolation. Considering this, the increased release of N from SOM by input of labile C and priming of microorganisms in acidic soils seems to be consistent, as well as an increased proportion of nitrate leached into deeper subsoil could have a share in lowered exudation rates in greater soil depth. Along the elevation gradient, a relationship between N availability and exudation rates was observed in comparable soil types on similar bedrock. Exudation rates were negatively correlated with N and C content, but not with the soil C : N ratio, for which no differentiation was made

between mineral and organic N in this study. We would have expected lower mineral N with increasing elevation, justified by lower temperature effects accumulating with low pH. This would have been a consideration for higher exudation rates, but apparently such a relationship is masked by other factors.

Fine root biomass in the topsoil had the significantly highest contents on more acidic soils, consequentially the annual exudate flux was higher there. Significantly decreasing total fine root biomass and a change in root morphology from rather fine, fibrous to pioneer roots, characterized by higher density, diameter, and lower relative surface area were observed with increasing depth at the glacial deposits site. Furthermore, low SRL values were found in the topsoil of sites with loess and basalt over medium to high SRL on glacial deposits, this was accompanied by a decrease in diameter. The increase in SRL was concomitant with an exponential increase in exudation rates, suggesting a relationship of root morphology, especially SRL to exudation of beech across different soil types.

A basal, passive outflow of exudates follows the gradient between root and soil solution and membrane conductivity (Jones et al., 2004). To limit this basal flux in unfavourable regions, a morphological adaptation of the fine roots appears to be a purposeful adaptation. Pioneer roots with less relative surface area and fewer branches, and thus fewer root tips, provide less metabolic active areas for exudation. Plasticity in root systems was discussed by Yanai et al. (1995) as a strategy to optimize the acquisition of resources in relation to different environmental conditions. Thus, there is evidence for both, first the expansion of the root surface area, and consequentially the metabolically active areas, for effective utilization in more nutrient-rich regions or those with minable nutrients from SOM, via priming of microorganisms, and second for effectively adjusting losses through passive outflows depending on the environmental conditions. Accumulating the majority of FRB in the significantly nutrient-richer topsoil rather than the poor subsoil (Chapter 2) could also be interpreted as a mechanism to effectively restrict C losses in uninhabitable regions. The accompanying reduction in microbial biomass could be seen as a consequence of low rhizodeposition and the availability of effectively usable SOM. The decrease in enzyme activity accompanied by the reduction of fungal biomass in acidic sites could indicate an increased need for exudates to trigger priming effects by non-fungal microbia to fill the gap formed by the lower abundance of fungi. On the other hand, there might be an alternative pathway on the less acidic soils. Assimilates and metabolites reaching the root tissues are partially extracted by the fungal symbiont via Hartig's network. Due to the comparably higher fungal abundance, it can be assumed that a much larger proportion of the exudates is extracted

and to a lesser extent available for rhizodeposition by the plant, rather to be used by the fungal symbionts and subsequently for the formation of fungal exoenzymes. Based on this argumentation, reduced plant exudation in less acidic soils should have to be considered in relation to the substances deposited by the fungal symbonts in order to assess both the C input in the soil and the magnitude of priming effects. However, there is the difficulty of estimating whether and to what extent the difference in soil fungal biomass also includes the ECM-forming fungi. Another explanation for increased exudation rates in soils where fungal biomass is reduced could be that fungi can access more distant nutrient deposits with their elongated hyphae (Fontaine et al., 2011). If the fugal proportion is smaller, raised rhizodeposition might lead to increased microbial turnover and radius of action of exudates and possibly of the rhizosphere, which would be particularly beneficial with observed increased FRB.

Some studies (Hutchings & de Kroon, 1994; Mou et al., 2013) found no or only minimal differences, when comparing fine root morphology along heterogeneous nutrient availability or soil chemistry.

This partially contrasts our findings along the nutrient availability gradient (Chapter 3), whereas similar results were provided by the elevation transect (Chapter 4); increasing nitrogen content with rising altitude, as well as rising acidity showed no relationship to fine root morphology, but exudation rates were related to N. Perhaps in this case the morphological patterns are less dependent on soil chemistry and nutrient availability (after crossing a certain threshold level) but reach a certain phenotypic expression to create the potential to trigger nutrient release via exudation.

In addition to a basal, passive outflow, the additional fraction of the flux can possibly actively restricted on the one hand, but on the other hand also be adjusted either directly as a result of environmental factors, that influence photosynthetic rates (Kuzyakov & Cheng, 2001) or by being a C surplus that is formed under better photosynthetic conditions but cannot be used for growth due to other limitations and has to be disposed (Prescott et al., 2020). Morphological adjustments to limit such losses might be too costly compared to the losses from exuded carbon itself.

This could be interpreted as a kind of cost optimisation, as possibly the benefit of reduced losses by exudates does not compensate for morphological adaptations.

Farrar & Jones (2000) reviewed four theories about the control of carbon acquisition to roots; a functional equilibrium theory, `push´ and `pull´ theories and the general or `shared control´ hypothesis, which place differing emphasis on the conditions and characteristics of the

aboveground plant part, or those of the roots, as the factors determining the magnitude of the assimilate influx into the root, or the variables affecting both at the same time, in proportion to the effects they excert on both plant parts. The present study rather supports the `shared control´ hypothesis according to the above approach, as both, the variable assimilate production and resupply might influence C translocation to the root, as well as rhizosphere processes influencing the exudation amount (i.e. priming of N mining microbes). For example, morphological adaptation to soil conditions with increasing depth, which is at least in our study strongly related to the amount of exuded C and surrounding soil conditions, can be seen as a factor influencing the influx of assimilates into the root and further their outflow, while the influence of temperature affects both photosynthesis and exudation rates, possibly directly in the form of accelerated diffusion, among others. The control of the C flux seems to be shared partly between the many processes controlling the root and the shoot.

Using the results from this study, an assessment of exudation in the subsoils of the other sites along the bedrock gradient can be cautiously made using other results from this project. A study from the same project (Kirfel et al., 2019) showed less pronounced differences between FRB of limestone derived topsoil and subsoil (44-58 % of topsoil FRB) compared to acidic soils (11-20 % of topsoil FRB). Similar variations were observed for SRL in acidic soils (ca. 12 % of topsoil), limestone (20 % of topsoil) and Loess (29% of topsoil). This was accompanied by much lower decreases of C : N ratios or even weak increases with raising depth in the subsoil of limestone and basalt and comparatively a 50 % to a one-third reduction in the C : N ratios in the subsoil of the acidic sites. The subsoil on limestone, compared to the more acidic sites, had a much higher percentage of SOC (ca 55 % of topsoil), similar results were observed for loess and for basalt, which dropped to ca 30 %. In contrast, the content of acidic soils declined to about 10% towards the subsoil (Kirfel et al., 2019). To estimate the proportion of root exudation to belowground carbon cycling, the Relation between SRL and Exudation, which could be observed as substantial, as well as given C : N ratios, may contribute. The relation between SRL and exudation under certain environmental circumstances may help to assume subsoil carbon fluxes on different parent materials by comparing local subsoil SRL values, FRB accumulation and C : N ratios as an approximation for minable N. The smaller decrease in SRL in the subsoil of the more base-rich sites compared to the glacial deposits, in addition to the higher FRB content at depth, suggests that more roots with greater proportions of metabolically functional areas are present in the subsoil, which may indicate a partial shift of exudation and nutrient uptake to depth.

Lower C : N ratios and the distribution of FRB suggest a more homogeneous distribution of

minable N in the subsoil, although the sole consideration of FRB distribution for nutrient estimation is disputed by some authors (Guo et al., 2008). Both the C : N ratios as well as the amount of SOC indicate minable contents of SOM in the subsoil of these plots, in contrast to the acidic soils of the other plots, whose SOM accumulates mostly in the topsoil. This should influence both the local microbial biomass in abundance, distribution and composition and subsequently the amount of exudates released in the assumption of priming effects. Higher exudation rates can be assumed in the subsoil of limestone, loess and basalt in comparison to results for glacial deposits conducted in the present study. Following the distributions of SOC and C : N in the subsoil, the C fluxes could be more homogeneously distributed in these subsoils. This could be a way to prevent local nutrient scarcity in the topsoil of these sites, where fresh litter, as a major nutrient source, is decomposed much faster, and thus a short-term higher competitive situation can be assumed. This is in contrast to acidic soils, where nutrient acquisition is much more centralized at the sites of nutrient input by slower litter decomposition in the topsoil, and where the subsoils are usually limited in nutrients, lacking as an alternative nutrient source.

Microbes in the subsoil are mainly limited by a lack of fresh organic carbon (Fontaine et al., 2007). This creates dependencies on the input of labile C; an effect that might be intensified in subsoils with lower C : N ratios in terms of priming effects and correspondingly higher bacterial biomass, and should increase the proportion of exuded C there. Salomé et al. (2010) found in a nutrient rich Eutric Cambisol, that fast-growing microbial communities in the subsoil, in contrast to the topsoil, where they were adapted to much more complex compounds, preferentially used simple molecules of the types commonly present in exudates. Thus, shifting exudation for the purpose of resource acquisition proportionately to the subsoil, may mean more assimilate investment, added to the effort of forming the root system deeper and wider. This could be compensated by benefits such as avoiding competition from other species that are more abundant in the understory of base- and nutrient-rich sites.

Relationships between temperature, root exudation and C and N in soils

Climate change reports and projections present different scenarios, but they usually predict an increase in temperature and a decrease in precipitation for Central Europe in addition to rising amounts of CO_2 in the atmosphere. A variety of studies dealt with interactions of fine root traits and rhizodeposition under CO_2 increase (Iversen, 2010; Kuzyakov et al., 2019; Langley et al., 2009) drought (Leuschner, 2020; Liese et al., 2017; Nikolova et al., 2020; Preece et al., 2018; Williams & de Vries, 2020b) or temperature increase (Štraus et al., 2015; Yin, et al., 2013a; Yin, et al., 2013b; Zhang et al., 2016). Fine roots are an important trait for describing the magnitude of C input into the soil; in particular, the proportion of root exudation under changing climatic conditions is discussed (Uselman et al., 2000). Several studies, published in the last decade, have addressed the interplay of artificially elevated temperature, root exudation, and SOM decomposition and N mineralization (Qiao et al., 2014; Yin et al., 2013a; Yin et al., 2013b; Zhang et al., 2016). A comparison to the present study is possible with limitations, since the studies were conducted mainly with seedlings of Gymnospermae, but *in situ* studies in mature tree stands are scarce. A clear correlation between artificial heating over longer periods of time, increased quantity and changed quality of released root exudates and increased N mineralization in the soil could be shown (Zhang et al., 2016). Furthermore, evidence was collected that higher N requirements of the plant must be met during warming, in the case of the cited study either by available fertilizer or by increased exudate-mediated N mining from SOM (Yin et al., 2013a). The availability and utilization of N is of special importance with respect to warming and corresponding adjustment of N acquisition strategies including adjustment of fine root morphology (Yin et al., 2013a). In the elevation transect study (Chapter 4), a relationship was found between mean annual temperatures of individual stands and root exudation for beech forests studied *in situ*, but much more prominent was the relationship to short-term temperatures prior to sampling. This was accompanied by a significant reduction in topsoil organic layer thickness and mass, soil nitrogen content and fine root biomass in the organic layer with increasing mean temperatures, while there were no relevant adjustments in fine root morphology. The accumulation of the organic layer FRB, following decreasing temperatures, suggests efficient utilization of this supply of organically bound nutrients. The high similarity of root morphology along the transect, compared to mean summer temperatures, contrasts with results from artificial warming studies, which showed a significant increase in length with temperature (Yin et al., 2013a). An interpretation might be, that under given comparable soil

conditions, the plastic formation of fine roots settles into an optimized use shape in consideration of variable climatic regimes at various altitudes of natural beech forests, which consequently differs from the plastic form that occurs under constant artificial warming. The increase in exudation rates in response to short-term temperature regimes in natural stands might be understood as a rapid adaptation. The climate especially in mountainous locations is characterized by high variability, and the necessity for rapid and efficient utilisation or responsiveness capacity to beneficial short-term conditions appears plausible. Allocation of assimilates to the root occurs very rapidly, sometimes within a single day, as shown by experiments with labeled (^{13}C and ^{14}C) CO_2 (Pausch & Kuzyakov, 2018), which provides an explanation for the increased root-borne C outflow as a rapid reaction.

This could be either a direct consequence of altered photosynthetic activity as a result of higher solar radiation, which in the given climatic region, is associated with elevated temperatures, and whose assimilate bonus is available for the roots and subsequently exudation, or as a consequence of increased nutrient demand, resulting from raised photosynthetic capacity, which could be dealt with by means of exudation and priming.

The relationships between nitrogen mineralization rates and both exudate quantity and quality were shown by Zhang et al. (2016). Exemplary bacterial strains involved in the mineralization of nitrogen were treated with root exudates obtained from seedlings under warmed growth conditions; in addition, the exudates were analysed for their composition. The results revealed both an increased C : N ratio (at constant N) of exudates obtained under warming treatment, as well as a significant promotion of bacterial growth, which was absent in the non-heat treated control. This indicated an increase of nitrogen-poor carbon compounds in the exudates, which apparently had promoting influence on the bacteria of nitrogen mineralization. If taken as a basis, despite the mentioned methodological limitations, then these relationships, transferred to the beech forest elevation transect, suggest that during higher temperature phases, exudation triggered N mineralization increases. Consequently, those plots that are lower in elevation and exhibit more frequent warmer temperatures should reveal significantly higher exudate-induced N mineralization. Therefore, lower stands are more N limited, a) by higher N demand by trees as a result of increased growth due to warmer conditions, b) by higher exudate-mediated N mineralization and consecutive absorption by trees, and c) generally increased temperature-mediated SOM decomposition and N release.

However, when considering temperature relationships and root exudation, the influence of seasonality should also be considered. Plants exude a remarkable portion of their assimilates by the roots (Canarini et al., 2019). Presumably, there are seasonal reductions in exudation

rates because beech has a peak photosynthetic capacity in the summer months (Schulze, 1970). Even though the exudation rates did not show significant correlations with respect to seasonality in relation to the depth gradient (Chapter 2), the lowest topsoil exudation rates were still measured in October 2015, comparable late-seasonal measurements did not occur along the bedrock gradient (Chapter 3), nevertheless, statistical analysis revealed, that sampling date had significant influence on the amount of exuded C. Seasonal effects were also observed along the elevational gradient (Chapter 4). The last sampling date in September 2015 provided the lowest exudation rates, which were especially low in altitudes above 400 m. These findings may point to seasonal reduced photosynthetic capacity and therefore decreasing assimilate proportions allocated to the roots.

Main Conclusions

In this study, three subprojects were conducted to investigate the relationships between different ecological factors of edaphic and climatic type and relations to the quantity of root-derived exuded carbon. The focus laid on the analysis of exudation rates, root morphology and nutrient conditions with increasing soil depth, along a nutrient gradient based on soil types originating from different bedrock materials, and furthermore an altitudinal transect, which brought in the additional consideration of differing climate.
The following conclusions can be drawn from the present study:

I. A close correlation between fine root morphology and the amount of exuded carbon was observed. Increasingly finer and more fibrous roots exuded higher amounts of C due to an increased proportion of metabolically active root areas. The observed dependencies allow conclusions to be made regarding the magnitude of priming effects. Fine fibrous roots condition the possibility to exude a sufficient quantity of C, which in turn provides the basis for the order of magnitude in which the priming of microorganisms is at least possible.

II. Soil regions with higher proportions of organic-bound N exhibit, in addition to accumulated fine root biomass, morphologically finer roots with increased length and surface area, as well as higher exudation rates.
These adaptions relate to the presence of minable soil organic matter and microbial biomass. In soil regions, where effectively minable SOM is minimal or nutrients are easily available

with limited necessity of microbial nutrient mining, beech trees adjust these root-related criteria for the purpose of optimization.

III. The study of soils deriving from different bedrock sources with distinct chemical properties revealed, that acidic, N poor soils were characterized by a lower proportion of fungi in the microbial biomass and lower activity of fungal derived exoenzymes that degrade poorly bioavailable carbon, while exudation rates increased. The nutrient mobilization gap due to lower enzymatic activity seems to be compensated by a higher proportion of exuded C to the surrounding small-scale ecosystem of the rhizosphere.

IV. Relationships between long-term site-specific climate and exudation rates were documented but were weaker than measured connections to the short-term temperature regime, which was highly significantly correlated to the amount of exuded carbon, indicating a lesser importance of the whole climate regime on root derived carbon fluxes.
This could be interpreted either as an adaptation to the climate-induced fluctuations in the tree's assimilate production, resulting in either an assimilate gain caused by useful climate with rapid allocation and excretion in the form of exudation (disposal) or a nutrient demand following the increased photosynthetic capacity, which is met by means of acquisition via exudation and priming. Exudation appears to be a mechanism of short-term adaptation to respond quickly to emerging surplus or deficits.

Further research recommendations

In the course of these studies, some open questions and approaches have emerged that could be included in future studies.

1) The relationship between exudation and soil nitrogen availability should be further investigated. The differentiation of mineral and organic bound N in the soil, under quantification of priming effects, in relation to exuded C could present given relationships more accurately.

2) The depth profile approach should be investigated further with the inclusion of soils from the nutrient availability gradient to study rhizodeposition in subsoils. Root

exudate sampling should be conducted from the subsoil of different soil types and quantitatively and qualitatively investigated, to study, how root derived carbon fluxes get altered in dependency of variable nutrient availability and spatial distribution as well as soil chemistry, and how the composition of exudated organic compounds is adjusted to different microbial conditions.

3) Investigations of temperature dependencies of root exudation should be continued and extended. The qualitative investigation of exudates with respect to their N content and simultaneous determination of N mineralization rates in the soil could allow conclusions on the contribution of root-borne N mobilization via priming microorganisms. A higher frequency of measurements over the whole growing season could cover the range of seasonality, the gradual investigation of further tree species, e.g. *Picea abies* would allow interspecific comparisons. In order to achieve better estimate of the effects of short-term climate on photosynthesis and in the end also the amount of C released by the fine roots, the additional measurement of light radiation, at least photosynthetic active radiation, and photosynthetic rates in addition to the determination of temperatures seems to be purposeful.

Summary

The availability of mineral nutrients (i.e. N, P) in unfertilized soils is often not adequate to meet plant demand, which in turn creates a necessity to fulfil the supply requirements from nutrient release from soil organic matter (SOM). SOM has a high chemical complexity, and it varies in terms of temporal and spatial availability. It contains essential nutrients in heterogeneous composition and distribution (Lal, 2009; Murphy et al., 2015; Schmidt et al., 2011). Root exudates are a key component of the mechanisms for acquisition and mobilization of nutrients in the soil. Their influences can be classified as both direct, i.e. the compounds released react directly by degrading soil material, or indirect, which requires intermediate steps of biological or physical nature, which in turn trigger effects. Direct effects are triggered, for example, by organic acids that raise P availability by complex alteration (Jones & Darrah, 1994; Jones, 1998; Bais et al., 2006; Eldhuset et al., 2007). Indirect effects are mostly priming effects or the modification of soil chemistry like acidification. Priming effects describe a short-term change in SOM decomposition rates as a response to an input of labile carbon (C) from living roots (Kuzyakov et al., 2000). The `nutrient-mining' theory presumes that microbial growth is promoted by the input of exogenous C and, as a consequence, a limitation of other nutrients such as N, raising the microbial decomposition of SOM and the mobilization of limited nutrients (Blagodatskaya & Kuzyakov, 2008; Wang et al., 2015).

Effects in the soil caused by the inflow of root exudates have been object of a multitude of studies, in contrast, the causative factors determining the amount of C exuded were poorly examined. Aiming to investigate influences of soil nutrient distribution, availability and climatic conditions on root exudation, three projects have been conducted, each using different gradual approaches. In a first project, investigations were carried out along a depth gradient in a soil near Nienburg (Weser), deriving from glacial sediments. Root exudate quantity, root morphology and architecture, soil chemistry and nutrient availability, different soil carbon classes and microbial biomass C were investigated. As part of a second project, beech forests were chosen along a nutrient availability gradient, containing different soil types, that originated from different bedrock material but were characterized by comparable climate. Measurements of the amount of released C by exudation and annual C fluxes, root morphology and biomass, parameters of soil chemistry and nutrient availability as well as fungal biomass and exoenzyme activity were determined. Both projects were associated to a larger research project, `SUBSOM' dealing with organic matter storage and turnover in subsoils.

The third project addressed an elevational gradient located in northern Hesse, Germany, with beech forests ranging from 300 to 800 m a.s.l., that had comparable soils deriving from bedrock material with similar properties but was characterized by distinct climatic differences. Climatic factors, particularly short- and longer-term temperature regimes, were documented, as well as root exudation rates, annual C fluxes, root morphology, fine root biomasses, soil chemistry and nutrient availability.

Root exudation decreased strongly with increasing depth; strong decreases in fine root biomass also reduced the annual flux per soil volume from topsoil to deep subsoil. Total N and P contents declined strongly with depth, accompanied by SOC and microbial biomass. Root exudation was related to EOC and N in topsoil but decreased to a minimal basal rate in subsoil. This may be viewed as a strategy of optimizing use efficiency, limiting carbon loss by exudation in soil regions where positive effects by priming are unlikely, but increased where SOM can be mined by microorganisms. Along the nutrient availability gradient, a negative relationship of exudation, org. C : N ratios and pH were found. In addition, increasing acidity led to decreasing proportions of fungal biomass and activity of enzymes that degrade poorly bioavailable carbon, while root exudation increased. This suggested, that in acidic, nitrogen-poor soils, root exudation increases while fungal abundance and activity decrease, and a greater proportion of assimilated carbon is invested by the plant into the surrounding rhizosphere.

Distinct relationships between root morphology and exudation rates were observed in both projects. With depth, the relative surface area and length of the roots became smaller, while tissue density and average diameter increased. Similar observations were made along the nutrient gradient, increasing relative length was associated with an exponentially raised amount of exuded C. Decreasing fibrosity reduces the functionally metabolically active areas of fine roots, resulting in reduced exudation. This appears to be a mechanism to restrict to basal exudation in regions where no benefit would be met. The elevation gradient provided results that indicate a highly significant relationship between the amount of exuded C and short-term temperatures. We could not find relations between fine root morphology and climate, while the long-term climate was related to the proportion of soil organic material and the mass of the organic layer as well as accumulation of org. layer FRB. The correlation between the short-term temperature regime and root exudation can be interpreted as a consequence of increased assimilate influx into the root and energetically accelerated higher outflow following the concentration gradient to the soil, as well as a consequence of increased priming of microorganisms in order to mobilize mainly N by exuding labile C according to demand.

Priming of soil microorganisms appears to be the most important function of root exudates. The presence of nutrients in microbially degradable form seems to determine the surplus of carbon released for direct purposes and the basal efflux.

In summary, the interplay between environmental conditions and fine roots is plastic at several levels. Abundance and morphological expression of fine roots occur in relation to local soil conditions, both soil chemistry and the pattern in which nutrients occur. Exudation appears to be a factor capable of compensating short-term nutrient needs and can be adjusted depending on the given morphology.

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Declaration of originality and certificate of ownership

I, Timo Tückmantel, hereby declare that I am the sole author of this dissertation entitled ‘THE INFLUENCE OF TEMPERATURE, NUTRIENT AVAILABILITY AND SOIL DEPTH ON ROOT EXUDATION IN EUROPEAN BEECH FORESTS’. All references and data sources that were used in the dissertation have been appropriately acknowledged. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure.

Göttingen, 14.02.2022 ----------

Timo Tückmantel