Hot or not? connecting rhizosphere hotspots to total soil respiration

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Abstract

Aims Soil organic carbon (C) efflux is tightly linked to the rhizosphere, where soil microorganisms rapidly decompose organic compounds released from roots. Recently, imaging approaches have greatly improved our understanding of small-scale C-turnover heterogeneity and promoted the term ‘rhizosphere hotspots’ for highly active areas. However, despite often assumed, the effect of these hotspots on total soil C balances is still unknown. We aim to bridge this gap by correlating rhizosphere imaging data to soil respiration on individual plant scale.

Methods We grew 17 maize (*Zea mays* L.) plants in rhizoboxes filled with sandy arable soil. After four weeks, the plants were labelled with $^{14}\text{CO}_2$ and root exudation was visualized and quantified by ^{14}C -imaging one day after labeling. The evolved CO_2 was trapped in NaOH and $^{14}\text{CO}_2$ as well as total CO_2 was quantified

before and after labelling. Enzyme activity (β -glucosidase) was quantified by soil zymography.

Results Bulk soil β -glucosidase activity negatively correlated to total CO_2 efflux, and was the most important predictor ($R^2 = 0.55$). Total and rhizosphere specific ^{14}C -activity were solely correlated to $^{14}\text{CO}_2$ efflux ($r = 0.51$, $r = 0.58$). A combination of bulk soil β -glucosidase activity, rhizosphere- ^{14}C activity and root biomass, explained about 75% of variance in CO_2 efflux.

Conclusions This indicates that root exudation and enzyme-activity hotspots are suitable predictors for total soil respiration, particularly when combined with root biomass to account for three-dimensional variation, and that hotspots on the rhizosphere scale are directly linked to larger scale C balances.

Keywords Zymography · ^{14}C -imaging · Soil-plant interaction · Root exudation · Root activity

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Introduction

Soils are not only providing the largest terrestrial carbon (C) pool (Lal 2008), soil CO_2 fluxes and belowground C allocation are also major pathways in the global C cycle (Ryan and Law 2005). Soil respiration (microbial & root) is responsible for most soil C losses and largely controls ecosystem C sequestration (Schlesinger 1997). In turn, it strongly depends on energy inputs from aboveground C sources. The major interface of C input and turnover is the rhizosphere, where soil

microorganisms rapidly decompose rhizodeposits (Hütsch et al. 2002; Gunina and Kuzyakov 2015). In contrast to the bulk soil, the rhizosphere is characterized by intensified C and nutrient turnover with high spatial and temporal heterogeneity along and across the root (Oburger and Schmidt 2015; Kuzyakov and Razavi 2019). Recent application of imaging approaches have greatly improved our understanding of this small-scale heterogeneity, and established the term ‘rhizosphere hotspots’ for particularly active areas (Kuzyakov and Blagodatskaya 2015; Kuzyakov and Razavi 2019). Despite often assumed, the direct relationship between these small-scale hotspots and soil C balances on the next higher scale level (i.e. plant individual) is still unknown.

Rhizosphere properties are controlled by root activity and by rhizodeposition (Jones et al. 2009). The size and extension of the rhizosphere have been well described (Kuzyakov and Razavi 2019). Low molecular root exudates are mainly released from the root tip and their concentration is highest within around 2 mm distance from the root surface (Holz et al. 2017; Kuzyakov and Razavi 2019). As they serve as easily available energy sources for microorganisms (Gunina and Kuzyakov 2015) and are linked to root growth (Holz et al. 2017, 2018), the occurrence of low molecular root exudates is likely associated with high CO₂ production. Usually, glucose is used as a model substance to simulate root exudation, commonly increasing CO₂ efflux rates after application (Blagodatskaya et al. 2011; Mason-Jones et al. 2018). This has established the assumption, that increased rhizodeposition generally results in higher microbial activity and soil respiration (Phillips et al. 2011). However, exudates not only consist of monosaccharides but include a broad variety of monomeric and polymeric substances. Chemically more complex root exudates (e.g. mucilage) and root necromass, are made bioavailable by microbially produced exoenzymes which break down polymeric components, such as cellulose, hemicellulose and other polysaccharides (Allison et al. 2011). Previous studies have reported positive relationships between enzyme activities and bulk soil CO₂ efflux (Phillips et al. 2011; Mancinelli et al. 2013). Therefore, it is assumed that this translates to spatially resolved patterns in the rhizosphere. Those areas with high activity of enzymes mediating SOC decomposing (i.e., enzyme hotspots) might also indicate areas with high CO₂ production.

Accordingly, concentrations of rhizodeposits and microbial biomass decrease from the root towards the bulk soil (Schenck zu Schweinsberg-Mickan et al. 2010; Holz et al. 2019; Kuzyakov and Razavi 2019). Major C and N turnover processes were found to be significantly higher in rhizosphere soil compared to the bulk soil (Finzi et al. 2015). However, such findings do not consider the spatial complexity within the rhizosphere, and do not allow for conclusion on the effect of rhizosphere processes on CO₂ efflux because both measurements are commonly not coupled. Quantifying CO₂ efflux alone does not account for process dynamics within the rhizosphere hotspots, while the quantification of rhizosphere hotspots alone might not translate to CO₂ balances on larger scale levels.

Assessing the importance of rhizosphere-imaging derived information for the C balances in plant-soil interactions, requires the application of flux measurements and imaging methods in unison. To bridge this gap between small-scale rhizosphere processes and individual plant-soil interaction, we applied different imaging methods on the root area of maize (*Zea mays*) plants grown in sandy arable soil. Root exudation was visualized by ¹⁴C-imaging (Pausch and Kuzyakov 2011; Holz et al. 2017), and β-glucosidase activity was imaged by soil zymography (Spohn et al. 2013; Razavi et al. 2016). The cumulative ¹⁴CO₂ and total CO₂ efflux rates were quantified over 1 day before imaging.

Our objectives were to assess (1) whether visible hotspots of root exudates and enzyme activity are a dominant source and thus suitable to predict total soil C respiration, and (2) whether it is possible to estimate the contribution of different rhizosphere processes to C respiration by coupling imaging and CO₂ flux measurements. We hypothesize that the intensity of root exudate and enzyme hotspots coincide with high CO₂ fluxes and that hotspots of rhizosphere activities are better predictors of CO₂ efflux than bulk soil or plant related variables alone.

Material and methods

Experimental setup

Rhizoboxes with an inner size of 20x40x1 cm were filled with an arable soil (each about 1 kg soil with a bulk density of 1.41) collected from a field close to

Göttingen, Germany. All boxes were treated the same to avoid clustering or covariate effects of confounding factors. Soil organic carbon content was 20.0 g kg^{-1} , total nitrogen was 1.7 g kg^{-1} and the $\text{pH}_{\text{H}_2\text{O}}$ was 4.9. Soil particle size was distributed as follows: Clay: 8.6%, silt: 18.5%, sand: 73% (Holz et al. 2017). Maize seeds (*Zea mays* - KWS 2376) were immersed in a 10% H_2O_2 solution for 10 min to avoid seed-borne diseases before germination. The seedlings were placed on a filter paper and were germinated in a petri dish 3 days in the dark. After 3 days, one seedling was planted per rhizobox ($n = 17$). During plant growth, the boxes were inclined by 55° to make the roots grow along the transparent front cover (Fig. 1a). This is relevant for measuring the distribution of enzymes around the roots during zymography and for ^{14}C imaging of roots and root exudates. The plants were kept in a climate chamber during the total growth period and watered daily to maintain a volumetric water content of 20–23%. The temperature was 25°C during the day, 22°C during the night, the photoperiod was 14 h, and the light intensity was $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$. These growth conditions were used as they represent optimal growth conditions for *Zea mays*.

^{14}C labelling and CO_2 measurements

After four weeks of plant growth each plant was labelled with $0.5 \text{ MBq } ^{14}\text{CO}_2$ (specific activity of $59.6 \text{ mCi mmol C}^{-1}$) for 5 h. Before starting the labelling, rhizoboxes were opened and a moist filter paper (Whatman, 1001–917, $11 \mu\text{m}$) was attached in order to capture exudates from the exposed root surface. After closing the rhizoboxes again, each rhizobox was packed in a plastic bag, which was closed with modelling clay at the lower part of the stem of the plants. Inside the bag, a $20 \text{ mL } 1 \text{ M NaOH}$ trap was placed to trap the $^{14}\text{CO}_2$ released from soil. For ^{14}C labelling, plants were placed in an acrylic glass chamber with a size of $0.6 \times 0.6 \times 0.8 \text{ m}$. A ventilating fan was used to distribute the $^{14}\text{CO}_2$ homogenously within the chamber. The label ($\text{Na}_2^{14}\text{CO}_3$ dissolved in 1 M NaOH) was placed into a glass vial, which was connected with the chamber by plastic tubes. After adding phosphoric acid (50%) to the label, $^{14}\text{CO}_2$ was released and pumped into the chamber where it was circulated for 5 h. 24 h after beginning of labelling the plastic bags were opened and the NaOH traps were removed for determination of total CO_2 as well as $^{14}\text{CO}_2$. The rhizoboxes were opened and the

moist filter paper was carefully removed from the rhizobox surface and oven dried at 60°C to avoid microbial decomposition of root exudates collected in the filter paper. Imaging plates (Storage phosphor screen, BAS-IP MS 2040 E, VWR, $20 \times 40 \text{ cm}$) were placed on the filter paper, which was covered by a thin plastic film for 14 h. After this, the screens were scanned (FLA 5100 scanner, Fujifilm) with a spatial resolution of $50 \mu\text{m}$ to visualize the distribution of ^{14}C in root exudates (hereafter referred to as “Rhizosphere ^{14}C hotspots” – Fig. 1b). After removal of the filter paper, the rhizobox surface was covered with a thin plastic film and a storage phosphor screen was placed on each rhizobox surface for 14 h. After this, the samples were scanned as described above to visualize ^{14}C allocated to roots and root exudates (hereafter referred to as “Total ^{14}C hotspots” – Fig. 1c).

To determine $^{14}\text{C}\text{-CO}_2$ in the NaOH traps a scintillation cocktail (Eco Plus) was added to NaOH with the ratio of 4:1. ^{14}C activity in NaOH was determined using a liquid scintillation counter (Hidex, 300 SL). Total CO_2 respiration from soil was measured from a subsample (2 ml) of the NaOH trap by a TOC analyzer (TOC 5050, Shimadzu Corporation, Kyoto, Japan).

Soil Zymography

The distribution of β -glucosidase activity was determined by soil zymography. The measurements were conducted according to Spohn and Kuzyakov (2013) but without a agarose gel as in Razavi et al. (2016). Briefly, polyamide membrane filters (Tao Yuan, China) with a pore size of $0.45 \mu\text{m}$ were cut into $20 \times 40 \text{ cm}$ pieces. Each membrane filter was placed in a solution of 20 mL of water containing $6 \text{ mg } 4\text{-Methylumbelliferyl-}\beta\text{-D-glucoside (MUF-G)}$ which is a substrate for β -glucosidase. After hydrolysis of the substrate, fluorescence of MUF can be measured. The membrane was soaked with the solution and was then attached to the rhizobox surface for 60 min. Subsequently, the membrane was removed from the soil surface and the remaining soil was carefully removed with a small brush. The membranes were placed under UV light, and pictures were taken at 360 nm wavelength (Fig. 1d) with a digital camera. In addition to the actual membrane, a small piece of membrane ($1.5 \times 6 \text{ cm}$) soaked with water instead of substrate solution was also imaged to control for the background signal. The final images had a spatial resolution of $40 \mu\text{m}$ per pixel.

Fig. 1 Exemplary images of (a) maize roots in an open rhizobox, (b) distribution of ^{14}C within the maize root system and in (c) root exudates imaged by phosphor imaging and (d) distribution of β -glucosidase activity in the same sample

For calibration, solutions with increasing 4-methylumbelliferone (MUF) concentrations were prepared: 0, 386, 967, 1934, 2901 and 3868 $\text{pmol cm}^{-2} \text{h}^{-1}$. Pieces of membranes ($4 \times 4 \text{ cm}$) were cut, soaked with the solution, and imaged as described for the soil zymography. From the amount of solution taken up per cm of membrane and the MUF concentration of the solution, the concentration of MUF per area was calculated. For the calculation of the β -glucosidase activity, the amount of MUF per area was divided by the incubation time. The equation obtained from the calibration was applied to all images to convert the grey value to β -glucosidase activity. The signal of the control membrane was subtracted from the image to remove the background signal.

Plant harvest

After zymography measurements, plant shoots were cut and dried at 40°C . The roots were taken out of the soil and the soil attached to the roots after being gently shaken (rhizosphere) was collected by carefully washing it from the roots. Roots were dried at 40°C . Rhizosphere soil and bulk soil (the soil not adhering to the roots) were freeze dried to avoid microbial degradation of labile carbon compounds. To measure ^{14}C activity, ground shoots, roots, rhizosphere and bulk soil samples were combusted in an OxyMAT OX500. The released $^{14}\text{CO}_2$ was captured in a scintillation cocktail (C400, Zinsser) and quantified using a liquid scintillation analyzer

(Tricarb, 3180, PerkinElmer). Shoot and root biomass was determined gravimetrically.

Image analysis

For quantification of ^{14}C in images, the images were converted from a log into a linear system by applying the following equation:

$$PSL = \left(\frac{Res}{100} \right)^2 * \frac{4000}{S} * 10^{\left(\frac{QL}{G} - \frac{1}{2} \right)} \quad (1)$$

Where PSL (photo stimulated luminescence) is the quantified value of the image in linear scale, Res is the resolution of the image in μm ($Res = 50 \mu\text{m}$), S is the sensitivity ($S = 5000$), L is the latitude ($L = 5$) and G is the gradation ($G = 65,535$). After conversion of the images, the background noise was removed: The part of the image where the screen was not in contact with the sample was selected and subtracted from the part of the image where the root system was visible to remove the background noise. Rhizosphere ^{14}C hotspots as well as total ^{14}C hotspots were identified based on the contrast between regions of high ^{14}C activity and regions with low ^{14}C activity and based on the longitudinal shape of the roots using the program RootTracker2D. A detailed description of the approach can be found in Menon et al. (2007). The hotspot area and intensity were calculated using

MATLAB (The MathWorks). Enzyme hotspots were also identified using the program Roottracker2D (Menon et al. 2007). For an objective selection of rhizosphere hotspots, rhizosphere extension was specified to the location where phosphatase activity had decreased to 5% of its maximum activity at the root center (Holz et al. 2020). As for ^{14}C imaging, hotspot area and intensity were calculated using MATLAB (The MathWorks).

Statistics

All data were checked for normal distribution and consistency, and measures of central tendency and dispersion were calculated (Supplementary Table 1). Variable interrelations were assessed by Pearson correlation and visualized by multidimensional scaling of the correlation matrix (network plot), where stronger correlated variables are ordinated closer to each other. Single effects of predictor variables (biomass and imaging parameters) on soil respiration (CO_2 and $^{14}\text{CO}_2$ efflux) were univariately assessed by simple linear regression. Residual diagnostics were conducted visually, as well as by using Shapiro-Wilk test and Cook's distance = 0.5. For all analyses the significance level was defined as $p < 0.05$.

We used random forest models (RF) to preselect explanatory variables for multiple linear regression. Variables with no, or negative % increase in mean square error were excluded. To avoid excess degrees of freedom due to missing values, ^{14}C activity in the rhizosphere was tested and compared iteratively. Variables in each multiple linear regression model were checked for variance inflation (VIC) and stepwise removed if $\text{VIC} > 10$. The akaike information criterion (AIC) was used to select the best performing multiple linear model. Models with better AIC values were only considered when significant ($p < 0.05$). The final model was visualized by partial regression plots, each showing the effect of one predictor variable on the response variable while other variable effects were kept constant.

All statistical analyses were conducted in R v3.6.1 (R Core Team 2017) using 'randomForest' package for building random forest model (Liaw & Wiener 2002), 'corr' package for correlation and network analyses (Kuhn et al. 2020), and 'ggplot2' for data visualization (Wickham 2016).

Results

Data verification and range

Except for shoot biomass, all predictor and explanatory variables followed a normal distribution among the boxes (Supplementary Fig. 1), and no data anomalies, such as outliers or clustering, occurred. The maize plants reached an average shoot drymass of 1.6 g (Supplementary Table 1). Their average root biomass was 0.67 g, ranging between 0.2 and 1.4 g, with a visible surface area between 0.8 and 8.2% of the whole rhizobox surface. From these boxes, we measured total CO_2 -efflux rates between 2 and 5 $\text{mg kg}^{-1} \text{ soil d}^{-1}$ (Supplementary Table 1). The cumulative $^{14}\text{CO}_2$ activity after 24 h ranged between 15 and 53 kBq. The ^{14}C -imaging provided a data range of 34,817 to 3,238,628 PSL for the total hotspots, and 25,537 to 145,708 PSL for the rhizosphere hotspots alone. The β -glucosidase activities in bulk soil ranged between 556 and 2541 $\mu\text{mol h}^{-1}$, while rhizosphere specific activities ranged between 5.16 and 114.93 $\mu\text{mol h}^{-1}$. The average areas of rhizosphere and bulk soil were 4.29% and 95.71% respectively.

The response variables (CO_2 and $^{14}\text{CO}_2$ efflux) were positively correlated to each other ($r = 0.41$, $p = 0.02$). Between the explanatory variables, significant interactions were detected (Fig. 2): The shoot and root biomass were directly related ($r = 0.80$, $p = 0.001$) and root biomass weakly correlated with total ^{14}C hotspots ($r = 0.46$, $p = 0.06$). The total number of ^{14}C hotspots strongly correlated with rhizosphere ^{14}C hotspots ($r = 0.72$, $p = 0.002$). No interactions were found between biomass variables and rhizosphere ^{14}C hotspots as well as enzyme activities. Correlations between ^{14}C -imaging variables and zymography variables were weak or completely absent.

Biomass related variables (i.e. root surface area, shoot and root biomass) showed strong correlation between each other, but were weakly related to CO_2 flux and imaging variables (Fig. 2). Zymography derived variables clustered with total CO_2 efflux, while ^{14}C imaging variables clustered with $^{14}\text{CO}_2$ efflux.

Variable effects on total CO_2 and $^{14}\text{CO}_2$ efflux

Zymography showed a clear negative effect of β -glucosidase activity on total CO_2 efflux (Fig. 3a, b). Bulk and rhizosphere enzyme activity each explained

Fig. 2 Network plot of Pearson correlations. Stronger correlations between variables are indicated by stronger paths (red = negative, blue = positive), and by appearing closer to each other (distances are determined by multidimensional scaling). Only correlations with $|r| > 0.4$ are shown and significance levels are indicated by * = 0.05, ** = 0.01 and *** = 0.001

more than 50% of the total CO₂ efflux variance. The ¹⁴C-imaging hotspots were not related to the total soil respiration, neither in the whole soil, nor in the rhizosphere specific area (Fig. 3c, d). The total CO₂ efflux was also independent from root surface area and biomass (Fig. 3e, f), although the latter showed a positive tendency ($p = 0.08$). In contrast to total CO₂ efflux, the measured ¹⁴CO₂ efflux was completely independent from zymography variables (Fig. 4a, b). The number of ¹⁴C activities in the total root zone and in the rhizosphere, however, were positively related to ¹⁴CO₂ efflux and explained 33% and 26% of the response variance, respectively (Fig. 4c, d). Yet again, the root biomass and root surface area had no effect on the efflux rate (Fig. 4e, f). The ¹⁴C activity of root-attached soil was unrelated to CO₂ or ¹⁴CO₂ efflux (Supplementary Fig. 2).

Variable importance

Root surface area, ¹⁴C activity of root-attached soil and shoot biomass showed either negative or no effects on RF predictive accuracy for total CO₂ as well as ¹⁴CO₂ fluxes and were excluded from further analyses. This equally applied for root biomass in ¹⁴CO₂ predictions.

The best performing models for predicting total CO₂ and ¹⁴CO₂ efflux from imaging methods alone, included enzyme activity and total ¹⁴C hotspots. This variable combination slightly increased the explained variance of total CO₂ efflux from $R^2_{adj} = 0.50$ (AIC = 25.9) to

$R^2_{adj} = 0.56$ (AIC = 25.1) compared to the best performing single explanatory variable (i.e. total enzyme activity). Adding root biomass as a 3rd predictor for total CO₂ efflux, strongly increased model performance ($R^2_{adj} = 0.75$, AIC: 18.8) (Fig. 5). The ¹⁴CO₂ efflux explained by rhizosphere-enzyme activity and total ¹⁴C hotspots was $R^2_{adj} = 0.65$ (AIC: 82.0), compared to $R^2_{adj} = 0.29$ by total ¹⁴C hotspots alone (AIC = 124.0). Combining enzyme variables to explain total CO₂, and ¹⁴C hotspots to explain ¹⁴CO₂ efflux, lead to lower R^2_{adj} values or non-significance due to multicollinearity effects, respectively.

Discussion

There is an emerging view that plants, and in particular the allocation of C to roots and rhizosphere, strongly control soil C cycling (Kuzakov and Cheng 2001; Hütsch et al. 2002; Jones and Hinsinger 2008). Rhizosphere-imaging approaches are powerful tools for spatially allocating hotspots of the major controlling processes (Oburger and Schmidt 2015; Roose et al. 2016). Yet, the overall importance of these hotspots for larger-scale C balances remains unclear. We used a combination of imaging approaches as proxies for root exudation and exo-enzymatic C turnover, to bridge the gap between rhizosphere-scale hotspots and soil C respiration on an individual plant scale.

Bulk soil β -glucosidase activities in combination with rhizosphere ¹⁴C activity and root biomass, explained up to 75% of total CO₂ efflux variance. This indicates that combining imaging hotspots of root exudation with hotspots of β -glucosidase activity allows to roughly predict soil respiration rates. Complementing these variables with root biomass measurements accounts for three-dimensional variability in the rhizosphere which may not be well depicted by 2D images. However, other variables – such as rhizosphere ¹⁴C-activity – did not seem to fulfil the same purpose.

In our study, root exudates were visualized through increased ¹⁴C-activity outside the root specific area. These exudates serve as easily available energy sources for microorganisms in the rhizosphere and are therefore expected to enhance SOM turnover and CO₂ efflux (Kuzakov and Cheng 2001; Hütsch et al. 2002; Gunina and Kuzakov 2015). This effect has been frequently reported, and is directly related to microbial activity and thus microbial respiration. Furthermore,

Fig. 3 Effect of β -glucosidase activities in bulk soil (a) and rhizosphere (b), ^{14}C -hotspots (c, d), and belowground-biomass variables (e, f) on total CO_2 efflux over 24 h. Significance level (p), coefficient of determination (R^2) and area of 95% confidence (grey) for simple linear regression are annotated

root exudation can be linked to root growth, i.e. is a proxy for fine root activity and root respiration (Sun et al. 2017). Both of these CO_2 sources can explain why increasing rhizosphere ^{14}C activity (i.e., increased root exudation) directly led to higher total soil respiration rates, when other variables were kept constant (Fig. 5).

In contrast to rhizosphere ^{14}C activity, β -glucosidase activities were negatively related to total CO_2 efflux and were the only variables to show significant univariate relationships. Given the equally negative correlations between β -glucosidase and ^{14}C activities, this suggests

that high root exudation leads to reduced enzyme activity, counterbalancing effects on total soil respiration. This contradicts our initial expectation, considering that β -glucosidase activity promotes SOM break down in bulk soil (Wick et al. 2002), and also contradicts the observation in previous studies where β -glucosidase activity was positively related to CO_2 efflux (Gispert et al. 2013; Liang et al. 2015). However, Gispert et al. (2013) also reported a negative relationship between β -glucosidase activity and CO_2/SOC ratio, indicating that these positive correlations may actually be artifacts of SOC to CO_2 relationships. Since our study design

Fig. 4 Effect of β -glucosidase activities in bulk soil (a) and rhizosphere (b), ^{14}C -hotspots (c, d), and belowground-biomass variables (e, f) on $^{14}\text{CO}_2$ efflux. Significance level (p), coefficient of determination (R^2) and area of 95% confidence (grey) for simple linear regression are annotated

Fig. 5 Partial regression plot for total CO_2 efflux explained by bulk β -glucosidase activity, rhizosphere ^{14}C activity and root biomass. Each trenline shows an explanatory variable effect when other variables are kept constant

(mostly) eliminates the impact of different bulk soil SOC contents, the negative β -glucosidase – CO_2 relationships might instead be a result of soil microbial reaction to substrate availability. Microbes predominantly invest in producing exo-enzymes for OM decomposition when labile C sources (such as root exudates) are scarce, leading to higher β -glucosidase activity and lower bacterial growth rate (del Giorgio and Cole 1998). Consequently, we found lower β -glucosidase activity in the presence of root exudates (i.e. rhizosphere ^{14}C activity) and a stronger relation between bulk β -glucosidase activity and CO_2 than between rhizosphere β -glucosidase activity and CO_2 . In turn, this suggests that zymography β -glucosidase hotspots might be associated with turnover of native SOC rather than plant derived C. This is supported by the strong relationships between β -glucosidase hotspots and total CO_2 efflux. In contrast, ^{14}C imaging variables were closely related to $^{14}\text{CO}_2$ efflux, emphasizing their importance for root, as well as rhizo-microbial respiration (de Vries et al. 2019).

Those variables that are directly dependent on the presence of plant roots (i.e. root surface area and root biomass) were weakly correlated to total CO_2 and $^{14}\text{CO}_2$ efflux, as well as to all imaging variables (Fig. 2). Because root exudation strongly varies along the root axis, with maximum intensities directly at the tip and shortly behind the tip (Dennis et al. 2010; Holz et al. 2017), exudation effect on CO_2 fluxes and related imaging variables are presumably decoupled from root biomass and root surface area. However, using solely imaging-derived variables to explain total soil respiration provided medium correlation strength and predictive power of simple, as well as multiple regression models, with just about half the variance explained. The inclusion of root biomass significantly increased the predictive power of the model. This might also indicate that a mediating factor is required to translate results from 2D-imaging methods to processes on a 3D-scale.

Conclusions

In this study, we used imaging methods to relate hotspots of root exudation and enzyme activity to total soil respiration on an individual plant scale. We conclude that these hotspots are reasonable predictors for soil CO_2 efflux and perform better than static variables, such as root biomass alone. Soil respiration was mainly

related to bulk enzyme activity (i.e., SOC turnover), while ^{14}C -hotspots mainly accounted for plant derived and rhizosphere $^{14}\text{CO}_2$ efflux. However, the relationships between hotspots and total soil respiration contain large uncertainties, and a combination of methods is required to achieve a meaningful representation of related processes. Further research is needed to evaluate these results concerning type, function and distribution of soil and rhizosphere hotspots as well as relationships to nutrient fluxes and root morphology. Increasing the range of values by including treatments, such as plant varieties differing in root exudation (Tawarayama et al. 2013; Holz et al. 2017) or fertilization levels, may also lead to stronger statistical relationships.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-021-04963-4>.

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Code availability Not applicable.

Authors' contributions Both authors have equally contributed to the manuscript and nobody who qualifies for authorship has been excluded.

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Data availability A data summary is available as an electronic supplementary. **Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11104-021-04963-4>.

Declarations

Conflicts of interest/competing interests The manuscript is the authors own work without any breach of copyright and the authors have no conflicts of interest to declare.

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