Soil Biology & Biochemistry 115 (2017) 213–220

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Mapping the footprint of nematodes in the rhizosphere: Cluster root formation and spatial distribution of enzyme activities

Bahar S. Razavi [a, *, 1], Duyen T.T. Hoang [b, c, 1], Evgenia Blagodatskaya [b, d], Yakov Kuzyakov [a, b]

[a] Department of Agricultural Soil Science, University of Göttingen, Göttingen, Germany
[b] Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany
[c] Department of Soil Sciences, Vietnam National University of Forestry, Hanoi, Viet Nam
[d] Institute of Physicochemical and Biological Problems in Soil Science, Pushchino, Russia

ARTICLE INFO

Article history:
Received 17 March 2017
Received in revised form
5 August 2017
Accepted 28 August 2017

Keywords:
Pathogenic nematodes
Meloidogyne incognita
Rhizosphere processes
Enzyme activity mapping
Zymography
Cluster roots
Nutrient acquisition
Microbial hotspots

ABSTRACT

Nematodes are among the most important pathogens in agriculture, greatly reducing crop biomass and yield. The direct effects of nematodes on above– and belowground plant parts are well known, but the broad range of indirect effects, especially on carbon (C) and phosphorus (P) cycles underground, remains unknown. For the first time, using soil zymography, we analyzed the indirect effects of *Meloidogyne incognita* cellobiohydrolase and phosphatase. The rhizosphere of lupine (*Lupinus polyphyllus* L.), a species sensitive to pathogens with high P demand, was selected to study the activity, distribution and localization of two enzymes responsible for C and P cycling: The distribution patterns of cellobiohydrolase and phosphatase demonstrated that *M. incognita* induced the formation of knots as well as cluster roots, which corresponded to hotspot locations on zymogram images for both enzymes. Increased C release by nematode–infected roots into the soil led to a decrease in the overall activity of cellobiohydrolase and especially at hotspots (by ~ 20 times). In contrast, the increased P demand of infected plants raised the phosphatase activity, leading to an increase in the rhizosphere extent around the roots and especially of the hotspot area (by 6 times). Remarkably, this 1 mm increase of rhizosphere extent in 2D equals a 2-fold increment in soil volume (3D) for nutrient mobilization.

We conclude that nematode infection not only has direct effects by changing root morphology, but also induces a number of subsequent biochemical changes (e.g. enzyme activities and consequently nutrient mobilization) in the rhizosphere, affecting C and P cycling.

1. Introduction

The complex interactions between plants and microorganisms in the rhizosphere – the soil volume affected by roots – are a compromise between costs and benefits. Microorganisms benefit from rhizodeposition and habitat niches, and plants inherit the available nutrients released by microbial community decomposition of organic matter. However, the C-rich rhizosphere (Bais et al., 2006) also attracts and accommodates pathogens. Among the various pathogens, nematodes – as the most abundant group of soil fauna – are a focus of interest (Ferris et al., 2001). Root-knot nematodes (*M. incognita*) are obligate biotrophic parasites that invade roots (Adam et al., 2014; Taylor and Sasser, 1978) by piercing and rupturing the cell walls with their stylets. This ultimately causes the cytoplasm to expand and become denser (Williamson and Hussey, 1996). Infected plants can be recognized after one month of infection based on the swollen root morphology. After invading the root, the infective juveniles either move down to the root tip, turning around the root apical meristem or migrate up to the root (Williamson and Hussey, 1996). To some extent, the whole root will be affected by a nematode infection, which leads to a change in root exudation (Bais et al., 2006). Root exudates are an important constituent of rhizodeposits, comprising carbohydrates, organic acids and amino acids (Bais et al., 2006; Hirsch et al., 2013). The composition of root exudates, however, is not identical along the entire root affected by nematode invasion (Hallmann et al., 2001). This is reflected in different microbial community compositions (Fontaine et al., 2007) and different activities of enzymes produced by both plant roots and microorganisms (Grierson and

* Corresponding author.
E-mail address: brazavi@gwdg.de (B.S. Razavi).
[1] Two first authors contributed equally to this work.

http://dx.doi.org/10.1016/j.soilbio.2017.08.027
0038-0717/

Adams, 2000; Blagodatskaya et al., 2009) along and around individual roots. Although the spatial distribution of enzymes is associated with root type (tap roots, fibrous roots) (Razavi et al., 2016), past studies have focused solely on healthy roots. However, the microbial community modified by ruptured plant cells and nematode activity can apparently regulate the activities of the respective enzymes in the rhizosphere. This is important, considering that species of *Meloidogyne* are parasites that secrete more than sixty plant cell wall-degrading enzymes, including cellulases, xylanases, polygalacturonases, pectate lyases and arabinases (Abad et al., 2008). Nonetheless, we lack any knowledge about the gradient or distribution of enzyme activities along and around individual roots in response to nematode attack.

Direct soil zymography – a non-destructive 2D technique – has been used to illuminate enzyme activities in soil (Sanaullah et al., 2016), biopores (Hoang et al., 2016), the rhizosphere (Ge et al., 2017; Razavi et al., 2016) and the detritusphere (Liu et al., 2017; Ma et al., 2017). This study presents quantitative imaging of enzyme activities in soil as a function of distance along and outward from the root to clarify whether 1) nematodes affect the spatial distribution of enzyme activities as a function of distance from the root; 2) this effect is enzyme specific. We conducted experiments on two enzyme activities – cellobiohydrolase and phosphatase – using the zymography technique. Cellobiohydrolase synthesized by microorganisms and nematodes (Williamson and Gleason, 2003) is a specific enzyme catalyzing cellulose degradation by hydrolysis of β-1,4-glycosidic bonds (German et al., 2011). Concurrently, nematode invasion shifts plants into a severe P-stress status (Venkatesan et al., 2013). In response, phosphatase is produced simultaneously by roots and microbes (Nannipieri et al., 2011) to increase P availability to cover the P demands of the plants. We hypothesize that i) cellobiohydrolase activity will be lower after nematode infestation due to more labile C (sugars) in the rhizosphere, whereas we expect a higher activity of phosphatase stimulated by the elevated P demand of plants; and ii) plant roots produce more P-acquiring enzymes in response to their P stress, caused by nematodes, leading to a larger spatial extension of phosphatase than cellobiohydrolase.

We installed rhizoboxes with nematode-inoculated and nematode-free lupine. Substrate-soaked membranes were applied to map the distribution of enzyme activities in the rhizoplanes. Image processing in Matlab was used to localize and evaluate enzymatic hotspots associated with root and/or nematode effects.

2. Materials and methods

2.1. Sample preparation

Soil samples were taken from an arable loamy Haplic Luvisol located on Campus Klein-Altendorf (50° 37′ N, 6° 59' E), south-west of Bonn, Germany. The soil consisted of 7% sand, 87% silt, 6% clay, with a bulk density of 1.4 g cm^{-3}, a water content of 30% at field capacity, a pH of 6.5, total C of 12.6 g C kg^{-1}, and total N of 1.3 g N kg^{-1} (Kramer et al., 2013; Pausch et al., 2013).

Eight lupine (*Lupinus polyphyllus* L.) plants were grown, each in a separate rhizobox with inner dimensions of 21.2 × 10.8 × 3.3 cm. The rhizoboxes were placed horizontally with front side open and soil was slowly and continuously poured into them through a 2 mm sieve to achieve a uniform soil packing and to avoid soil layering. The front side was then closed, the samples were turned to a vertical position, and they were gently shaken to achieve bulk density of 1.4 g cm^{-3} and a stable soil packing. The seeds were germinated on filter paper for 72 h. Then, one seedling was planted in each rhizobox at a depth of 5 mm.

Four lupines were inoculated– with the same level of inoculum–by aqueous suspension containing 50 *Meloidogyne incognita* after 10 days of plant growth and were incubated for 21 days before harvest. During the 31 days of growth, the rhizoboxes were kept inclined at an angle of 45° so that the roots grew along the lower wall of the rhizoboxes. Plants were kept in a climate chamber with a controlled temperature of 20 ± 1 °C and a daily light period of 16 h with a photosynthetically active radiation intensity of 300 μmol m^{-2} s^{-1}. During the growth period, the soil water content was maintained at 60% of the water holding capacity by irrigating the soil from the bottom with distilled water.

2.2. Direct zymography under influence of nematodes

After cultivating lupine for 31 days, zymography was applied as an *in situ* technique to study the spatial distribution of enzyme activities around the roots. We followed the optimized direct zymography method after Razavi et al. (2016). Enzyme activities were visualized using membranes saturated with 4-methylumbelliferone (MUF) substrates. Cellobiohydrolase activity was detected with 4-methylumbelliferyl-β-D-cellobioside (MUF-C), and acid phosphatase activity with 4-methylumbelliferyl-phosphate (MUF-P). Each of these substrates was separately dissolved to a concentration of 10 mM in MES buffer (Koch et al., 2007) (Sigma-Aldrich, Germany). Polyamide membrane filters (Tao Yuan, China) with a diameter of 40 cm and a pore size of 0.45 μm were cut to fit the rhizobox. The membranes were saturated with the substrates for each enzyme. The rhizoboxes were opened from the lower, rooted side and the saturated membranes were applied directly to the soil surface (Sanaullah et al., 2016; Razavi et al., 2016). After incubation for 1 h (incubation time was determined in preliminary experiments), the membranes were carefully lifted off the soil surface and any attached soil particles were gently removed using tweezers.

Quantification of zymogram images requires a standard calibration that relates the activities of various enzymes to the gray-value of zymogram fluorescence (i.e. of the membrane). The calibration function was obtained by zymography of 2 × 2 cm membranes soaked in a solution of MUF with concentrations of 0, 0.01, 0.05, 0.1, 0.5, 1, 3, 6, 8, and 10 mM. The amount of MUF on an area basis was calculated from the solution volume taken up by the membrane and its size. The membranes used for calibration were imaged under UV light and analyzed in the same way as the samples.

2.3. Image processing and analysis

Fluorescence of the zymograms under UV light shows the areas in which the substrate has been enzymatically hydrolyzed. The intensity of fluorescence is proportional to the activity of the enzyme. To obtain quantitative information, we processed the zymograms in Matlab according to Razavi et al. (2016). Briefly, zymograms were transformed into 16-bit grayscale images as matrices and corrected for light variations and camera noise. Then the zymograms were referenced based on the grayvalue received from a reference object embedded in all the zymograms. We used the grayvalue obtained from the blank sides (outside the membrane) of each image as the referencing point for all images. After referencing the zymograms, we calculated an average background grayvalue through the zymograms of calibration lines at zero concentration and subtracted this value from all the zymograms. Note that the same filters were applied to all of the images, including both the zymograms of the roots and the calibration baseline. The pixel-wise grayvalues from zymography were converted to enzyme activities using the calibration function obtained for both enzymes.

The resulting images were used for further analysis: four roots were segmented as replicates in each nematode-inoculated box, with roots clearly distinguishable from the surrounding soil due to strong contrast between the soil and roots. Therefore, we had a total of 12 roots from 3 nematode-inoculated boxes and another 12 roots from control boxes (without nematode infection). A threshold method in Matlab was used to detect the boundaries of the roots (Chaudhuri et al., 1989). The length and radius of segmented roots were calculated using the Euclidean distance map function in Matlab to calculate overall enzyme activity on root surface.

Hotspots were distinguished from the surrounding area by their contrasting color intensity in digital images. Based on referenced images and of the calibration line, the color intensity of all pixels exceeding the average value (i.e. >0.6) was assigned to hotspots of enzyme activity, represented by red color, with blue indicating lower activities (Hoang et al., 2016). To confirm the boundaries, one-way analysis of variance (ANOVA) was applied to assess the significant differences between independent variables (mean color intensity values of five adjacent pixels, i.e. equal to 0.1 mm). Significant differences between two adjacent groups of 5 pixels were then considered as a boundary for each category of activity (low, medium and hotspot) (Fig. S1) (Hoang et al., 2016). The ANOVA, followed by Tukey HSD test at a probability level of $p < 0.05$, confirmed the categories of enzyme activity and also defined the significant difference of one specific enzyme distribution between two treatments (with and without nematodes). Homogeneity of variance and normality were tested by Levene's test and Shapiro Wilk's W test.

3. Results

3.1. Overall enzyme activities and hotspots

The formation of root-knots and cluster roots by lupine was well pronounced one month after inoculation with *M. incognita* (Fig. 1). Consistently, for all replicates, inoculated plants showed the formation of 3 times more cluster roots, as well as more lateral roots, compared to the non-infected control. The zymograms of individual plants with and without *M. incognita* inoculation demonstrate the distributions of enzyme activities along and across the roots (Fig. 2 and Fig. 3). The percentage contribution of medium activity and hotspots to total activity of cellobiohydrolase decreased strongly (by a factor of 1.5 and 20 times, respectively) with nematode infection (Fig. 2). In contrast, these percentages for phosphatase increased by 1.2 times for medium activity and 6 times for hotspots (Fig. 3). High activity at root knots was a common pattern for both phosphatase and cellobiohydrolase.

Thus, nematode infection not only changed root morphology, with more generation of lateral and cluster roots, but also the overall enzyme activities on the rhizoplane and in the rhizosphere (Figs. 2 and 3).

3.2. Distribution of enzyme activities along and outward from the roots

Both enzymes demonstrated a similar uniform distribution along the roots (Fig. 4). However, cellobiohydrolase activities were 67% lower in infected versus control plants, whereas phosphatase activities were 56% higher.

The rhizosphere extent of non-infected plants varied between enzymes: the phosphatase activity distribution was broader (3–3.5 mm from the root) than for cellobiohydrolase (1–1.5 mm). The rhizosphere extent for both enzymes was strongly affected by nematode infection: phosphatase distribution was broader (4–4.5 mm) and cellobiohydrolase distribution was significantly narrower in infected plants (0.5–0.8 mm) than in healthy plants. Altogether, the radial patterns of enzyme activity around the lateral roots of infected plants differed from those for non-infected control plants (Fig. 5).

4. Discussion

M. incognita infection strongly affected the root morphology and rhizosphere biochemistry within 3 weeks of infection.

4.1. Root morphological response to M. incognita invasion

M. incognita infection had a clear effect on the host plant through root morphological changes and the appearance of root knots. The widespread appearance of knots along the main roots of

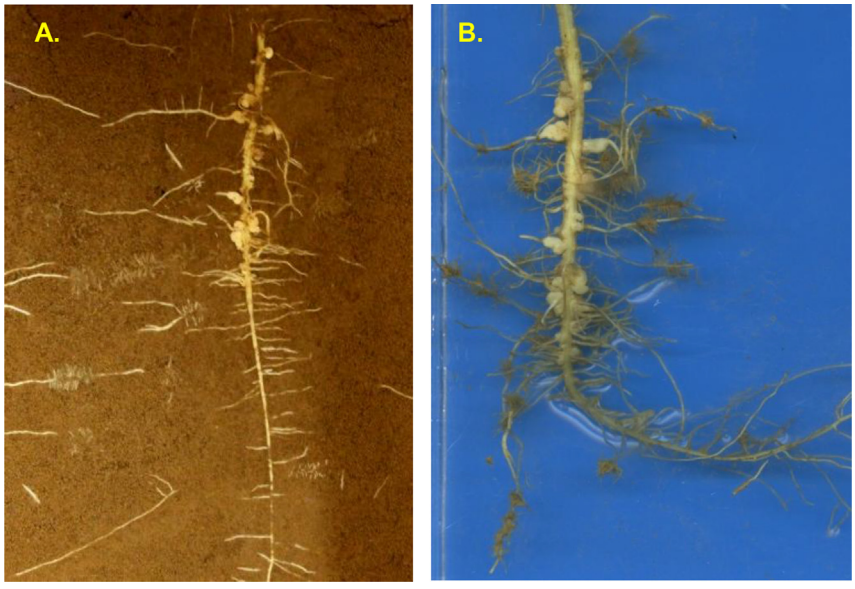

Fig. 1. A: Lupine 3 weeks after inoculation with *M. incognita*, B: Formation of cluster roots and position of root knots on main root are visible.

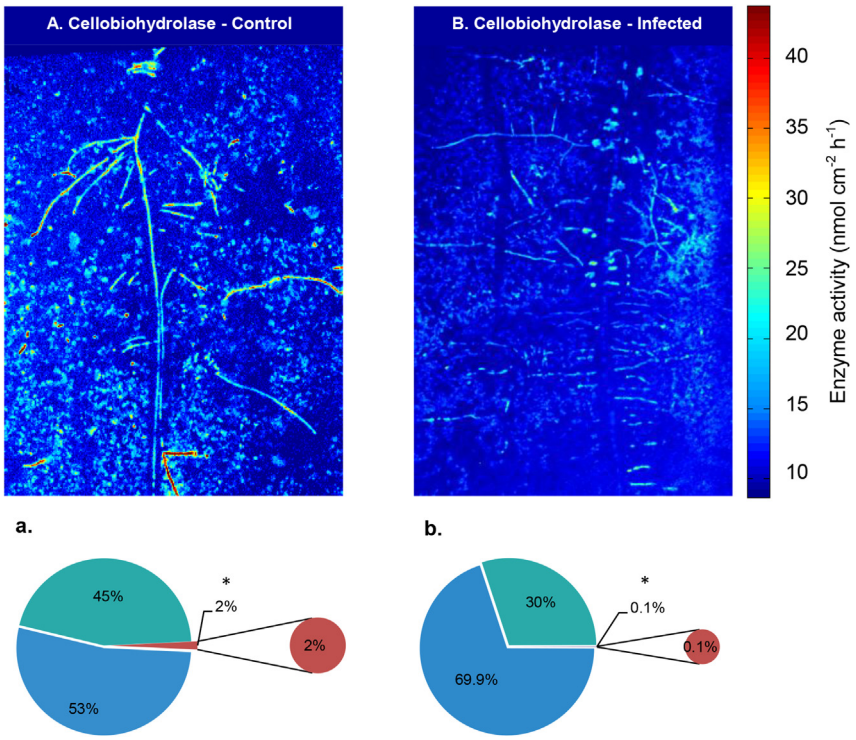

Fig. 2. Above: Zymogram of cellobiohydrolase activity: A) control (normal root) and B) infected with nematodes. Bottom: a. cellobiohydrolase hotspot percentage: control, b. cellobiohydrolase hotspot percentage: infected root. The color bar corresponds to enzyme activity (nmol cm^{-2} h^{-1}). Asterisks (*) indicate significant differences in hotspot percentage between control and infected plants at $p < 0.05$ after Tukey's HSD test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

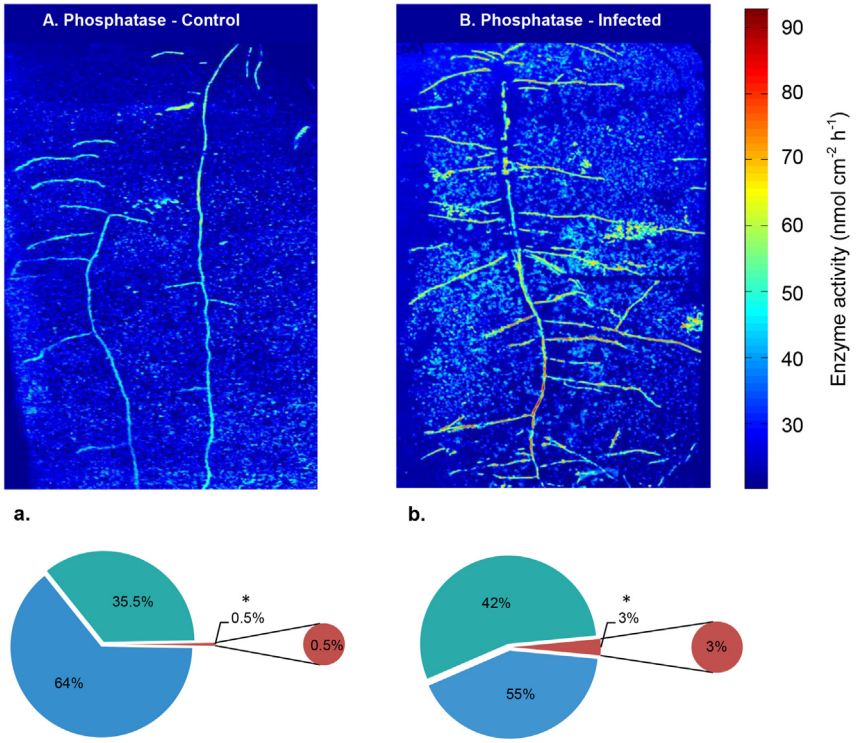

Fig. 3. Above: Zymogram of phosphatase activity: A) control (normal root) and B) infected with nematodes. Bottom: a. phosphatase hotspot percentage: control, b. phosphatase hotspot percentage: infected root. The color bar corresponds to enzyme activity (nmol cm^{-2} h^{-1}). Asterisks (*) indicate significant differences in hotspot percentage between control and infected plants at $p < 0.05$ after Tukey's HSD test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

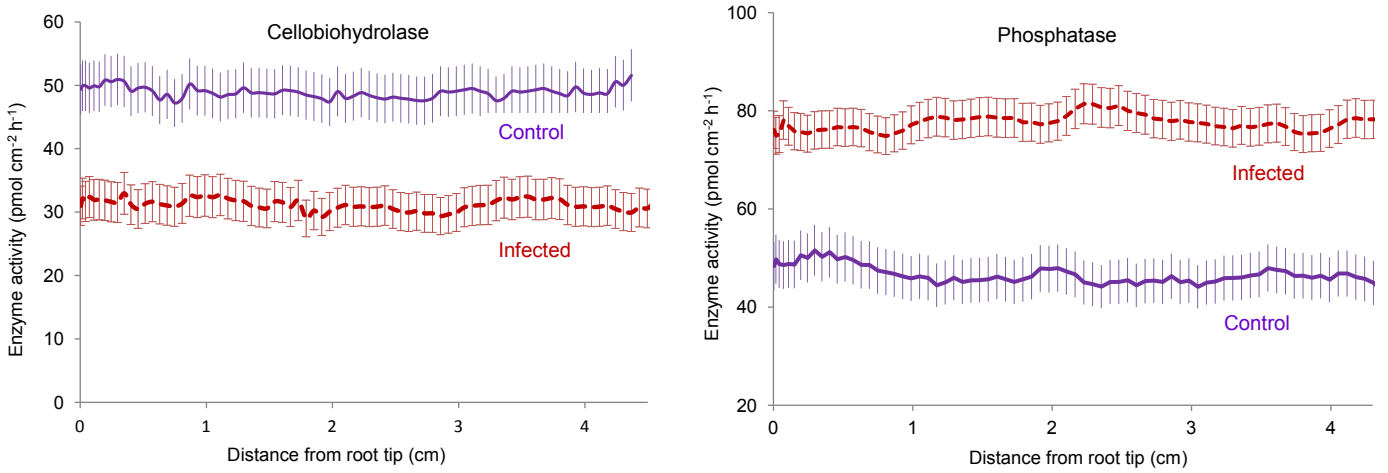

Fig. 4. Distribution of enzyme activities: Left: cellobiohydrolase and Right: phosphatase, along the normal and infected lateral roots.

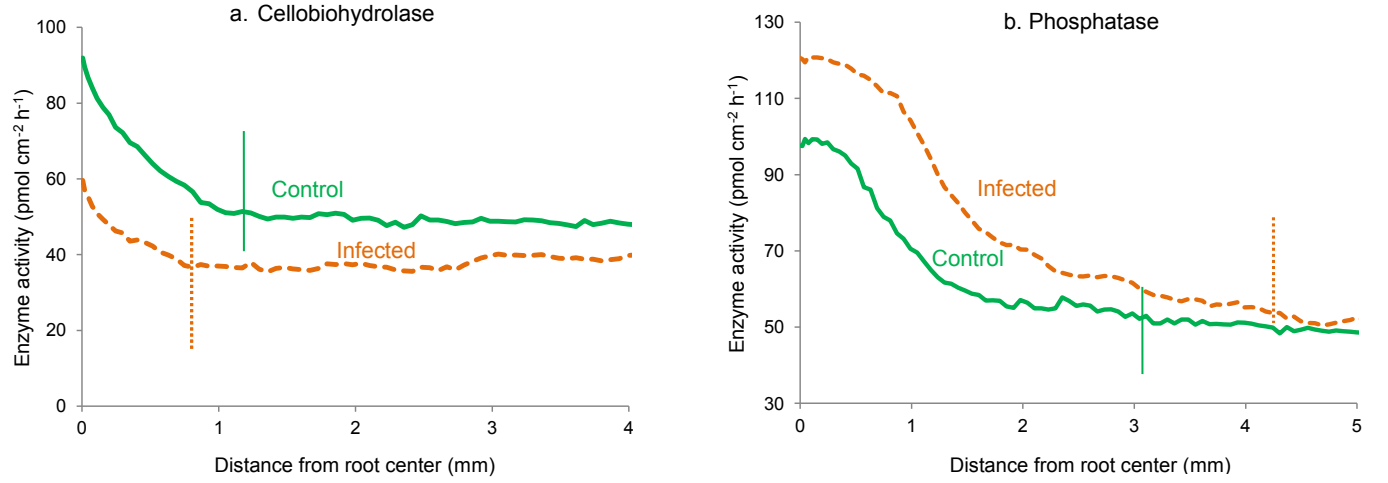

Fig. 5. Profile of enzyme activity distribution as a function of distance from the control (normal root) and infected lateral root center to the surrounding soil: a. cellobiohydrolase, b. phosphatase. Each line refers to the mean values of four sampled roots. Error bars are omitted to improve visualization. Bands indicate the rhizosphere boundary (extent) for each enzyme.

lupine is a clear symptom of nematode attack (Seinhorst, 1961). In contrast, an increased number of lateral roots and cluster-root formation are adaptations of the plant for nutrient acquisition (Neumann et al., 2000; Shane and Lambers, 2005). The interactions between roots and pathogenic nematodes lead to the reformation of root structure as a "signaling language" (Baron and Zambrysky, 1995; Mathesius, 2003). One of the first signs of nematode invasion is the local induction of lateral root growth at the feeding site (Karczmarek et al., 2004). In fact, the site of lateral root emergence is also a preferable site of penetration by nematodes (Bird and Kaloshian, 2003). Thus, the appearance of lateral roots is beneficial in terms of nutrient and water absorption by plants in response to nematode damage, but it also increases the possibility of pathogen invasion. Along with more lateral roots, cluster roots became more abundant in infected than in healthy plants in all replicates – this was the second signal of nematode infestation. Generally, cluster roots are stimulated by P starvation (Neumann et al., 1999; Gilbert et al., 1999). Our agriculturally used Luvisol is not P-deficient, so the generation of cluster roots was a nematode effect to increase the efficiency of phosphorus uptake by nematode-infested, P-demanding plants (Oteifa, 1952; Widdowson et al., 1972). Accordingly, cluster root presence supported the plant to deal with nutrient deprivation due to nematodes (Oteifa, 1952) by increasing the chemical mobilization of nutrients in the rhizosphere (Neumann and Martinoia, 2002) and by increasing its surface area for nutrient uptake (Marschner et al., 2002).

The release of root exudates attracts nematodes (Bird, 1959; Klinger, 1965; Clarke and Hennessy, 1987). Nematode feeding on roots, in turn, influences exudation (e.g. increased flux, altered chemical composition) and increases below-ground C allocation by the plant (Bonkowski et al., 2000). *M. incognita* not only stimulates the formation of more lateral roots (Evans and Stone, 1977), but also elongation of the root hairs (Haase et al., 2007) in response to low P availability (Lynch, 1995) and higher water and nutrient demand due to root cell disruption (Fig. 1). Thus, lateral roots increased by more than 2 times and cluster roots by 3 times after nematode infection. This demonstrates that the roots underwent pronounced morphological and physiological changes.

4.2. Hotspot distribution associated with roots, root-knot location and enzyme specifics

The differences in enzyme activities between infected and healthy roots demonstrated the quantitative and qualitative effects

of nematodes on rhizodeposition components, which is in line with findings by Haase et al. (2007). Our results consistently supported the first hypothesis that nematode infection decreases cellobiohydrolase activities but increases phosphatase activities (Figs. 2 and 3).

In the infected-plant rhizosphere, the enzymes are produced not only by roots and microorganisms, but also by nematodes (Abad et al., 2008). The multiple interactions among these organisms may restructure enzyme systems by changing C and nutrient availability near roots. The nematode effects were enzyme specific: decrease of cellobiohydrolase activities (cellulose hydrolysis) but increase of phosphatase activities (organic P hydrolysis). High and medium activities of cellobiohydrolase accounted for 30% of total enzyme activity in nematode-infected roots as compared to 45% in the controls (Fig. 2 bottom). The increased C leakage from infested roots (Yeates et al., 1998, 1999; Bonkowski et al., 2000), especially at the injury sites, diminished the production of enzymes involved in the C cycle. This is an energy-saving strategy of microorganisms (Denton et al., 1998; Bardgett et al., 1999; German et al., 2011). In contrast, the root knots are considered to be metabolic sinks (Wallace, 1974; Back et al., 2002), which attract fungi and bacteria. The colonization of plants by additional pathogens may lead to nutrition competition with the nematodes and enhance enzyme expression. High enzyme activities, therefore, were typical for both cellobiohydrolase and phosphatase at knot locations.

The production of cell-wall-degrading enzymes, namely cellulase (Williamson and Gleason, 2003), is the first step for nematodes to enter roots. This enzyme is also produced by microorganisms to hydrolyze cellulose in sloughed-off root cap cells. Therefore, hotspots of cellobiohydrolase (amounting to 0.1% of total activity around infested roots) represent the combined enzyme synthesis of nematodes and other microorganisms. These hotspots correspond mainly to the nematode knots (high color intensity in Fig. 2). Compared to healthy roots, cellobiohydrolase activities along the main root were lower, as indicated by lower color intensity. This is due to the accumulation of photosynthates at the nematode feeding sites (Bird, 1974; Bird and Loveys, 1975; Melakeberhan and Ferris, 1989) instead of being homogeneously distributed along the main root. High cellobiohydrolase activity at root knots was in line with the metabolic sinks proposed by Bird (1974) and Wallace (1974).

In contrast, hotspots and medium activities of phosphatase represented 42% of total activity in affected roots versus 35.5% in control soil (Fig. 3). This increase is explained by the 3-fold increase in plant P demand under nematode infestation (Widdowson et al., 1972). The P stress stimulates the rhizosphere microorganisms and roots (Tarafdar and Claassen, 1988; Miller et al., 2001) to produce more phosphatase along the whole root and especially at cluster roots. Such a proliferation of cluster roots increases the P uptake by 50% in white lupine (Neumann et al., 1999). Accordingly, high enzyme activity illustrated by high color intensity on zymograms, was associated with roots (Asmar et al., 1994; Marinari et al., 2014), nematode knots, and especially cluster roots. The phosphatase hotspots in infected plants had six-fold higher activities than those of healthy roots. Consequently, nematode infection triggered stress on the plant and heightened demand for P. In conclusion, the visualization of these two enzymes indicates the sites of root injury and the diverted translocation of C and P under nematode attacks.

4.3. Extent of enzyme activity along and across the root after nematode infection

The homogeneity of enzyme patterns observed for lupine corresponded to the pattern in lentil – another N_2 fixing plant (Razavi et al., 2016). We report new details about enzyme patterns in both healthy and infected roots.

Enzymes were continuously and uniformly distributed along the lateral roots (without knots or cluster roots) in infected plants and healthy plants (Fig. 4). This homogeneity is associated with the rhizodeposition pattern along the root (Neumann and Römheld, 1999) and suggests that nematodes do not change the pattern as such, but homogeneously increase exudation along the entire length of lateral roots. Furthermore, the homogeneous distribution of enzyme activities along lateral roots reflects the nutrient acquisition strategy of plants (Clarkson, 1991; Hinsinger et al., 2011).

The 2D-images (Figs. 2 and 3) as well as the enzyme activities along and across the roots (Figs. 4 and 5) consistently supported our hypothesis that the effect of nematodes is enzyme-specific. Cellobiohydrolase activities along the infected roots were 50% lower than in healthy plants. The increased availability of glucose and other sugars deterred microorganisms from producing enzymes involved in carbohydrate decomposition. Similarly, the distribution of cellobiohydrolase activity from the root center was narrower in contaminated versus healthy plants. Nonetheless, both infested and healthy roots showed the same decreasing pattern away from the root center, which steeply declined within 1 mm, becoming relatively stable outward into bulk soil. A 1 mm radius around the root belongs to that part of the rhizosphere with intensive exudation and rhizodeposition and is therefore a favorable habitat for microorganisms. The proliferation of bacteria and fungi induced the production of enzymes in this restricted area. Nonetheless, the narrower activity distribution of cellobiohydrolase (functioning in cellulose degradation) under nematode attack than in healthy plants supports earlier reports on the alteration of root exudate composition. The exudates of nematode-infected roots contained more water-soluble C (Van Gundy et al., 1977) and excessive sugars (Wang and Bergeson, 1974; Poll et al., 2007), which are available for microbial uptake. In this case, microbial communities were regarded as being economic units that maximize their productivity by allocating resources to extracellular pools of C-releasing enzymes, depending on substrate quality and nutrient availability (Sinsabaugh and Moorhead, 1994).

In contrast to the cellobiohydrolase distribution, infected roots showed a wider extension of phosphatase (4–4.5 mm) than non-infected roots (3–3.5 mm). Our interpretation is that, in response to higher P demand due to nematode infestation, the plants enhanced phosphatase production in their lateral roots. Thus, the infested roots, rather than microorganisms, play a pivotal role in producing phosphatase. Importantly, this 1 mm increment of rhizosphere extent in 2D equals a 2-fold increase in soil volume (3D) for nutrient mobilization.

Finally, as a next experimental step, we propose manipulating nematode infection as a tool to alter C fluxes and enzyme production to further our understanding of rhizosphere microbial activities.

5. Conclusions

Root infection by the nematode *Meloidogyne incognita* induced morphological and physiological changes in root tissues and thus biochemically altered the rhizosphere. These biochemical alterations involved increased C input due to root cell-wall rupture and increased plant demand for P. The result was profound changes in enzyme activities and localization (Fig. 6). The effect of the nematodes was enzyme specific: the increased P demand increased phosphatase activity, but the increased C supply decreased cellobiohydrolase activity. Moreover, the response of hotspots was also enzyme specific: nematode infection decreased the area and activity of cellobiohydrolase hotspots by a factor of 20. In contrast, the

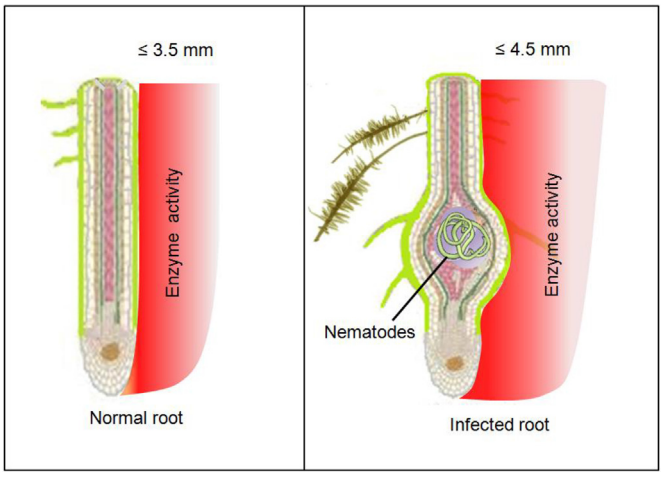

Fig. 6. Scheme of responses of root and enzyme activity to the nematodes infection.

phosphatase hotspots increased 6-fold. This correspondingly modified the rhizosphere shape of the enzyme activities across and along the roots. Using zymography to map the footprint of nematodes in the soil, we conclude that nematode infection not only has direct effects by changing root morphology, but also induces a number of subsequent biochemical changes in the rhizosphere, affecting C and P cycling.

Acknowledgements

We are thankful to Prof. Dr. Liliane Ruess for providing nematodes. The authors wish to thank Callum Banfield, Menuka Maharjan and Huadong Zang for field sampling. We gratefully acknowledge the Vietnamese government for supporting DH. Project was supported within the DAAD PPP Project 57316720. The study was supported by the German Research Foundation (DFG) by the project KU-1184/29–1 in the framework of the project PAK 888.1.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.08.027.

References

Abad, P., Gouzy, J., Aury, J.-M., Castagnone-Sereno, P., Danchin, E.G.J., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., Blok, V.C., Caillaud, M.-C., Coutinho, P.M., Dasilva, C., De Luca, F., Deau, F., Esquibet, F., Flutre, T., Goldstone, J.V., Hamamouch, N., Hewezi, T., Jaillon, O., Jubin, C., Leonetti, P., Magliano, M., Maier, T.R., Markov, G.V., McVeigh, P., Pesole, G., Poulain, J., Robinson-Rechavi, M., Sallet, E., Ségurens, B., Steinbach, D., Tytgat, T., Ugarte, E., van Ghelder, C., Veronico, P., Baum, T.J., Blaxter, M., Bleve-Zacheo, T., Davis, E.L., Ewbank, J.J., Favery, B., Grenier, E., Henrissat, B., Jones, J.T., Laudet, V., Maule, A.G., Quesneville, H., Rosso, M.-N., Schiex, T., Smant, G., Weissenbach, J., Wincker, P., 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature Biotechnology 26, 909–915.

Adam, M., Heuer, H., Hallmann, J., 2014. Bacterial antagonists of fungal pathogen also control root-knot nematodes by induced systemic resistance of tomato plants. PLOS One 9 (2). http://dx.doi.org/10.1371/journal.pone.0090402.

Asmar, F., Eiland, F., Nielsen, N.E., 1994. Effect of extracellular enzyme activities on solubilization rate of soil organic nitrogen. Biology and Fertility of Soils 17, 32–38.

Back, M.A., Haydock, P.P.J., Jenkinson, P., 2002. Disease complexes involving plant parasitic nematodes and soilborne pathogens. Plant Pathology 51, 683–697.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interaction with plants and other organisms. Plant Biology 57, 233–266.

Bardgett, R.D., Cook, R., Yeates, G.W., Denton, C.S., 1999. The influence of nematodes on below-ground processes in grassland ecosystems. Plant and Soil 212, 23–33.

Baron, C., Zambrysky, P.C., 1995. Notes from the underground: highlights from plant-microbe interactions. Trends in Biotechnology 13, 356–362.

Bird, A.F., 1959. The attractiveness of roots to the plant parasitic nematodes Meloidogyne Javanica and M. Hapla. Nematologica 4, 322–335.

Bird, A.F., 1974. Plant response to root-knot nematode. Annual Review of Phytopathology 12, 69–85.

Bird, A.F., Loveys, B.R., 1975. The incorporation of photosynthates by Meloidogyne javanica. Journal of Nematology 7, 111–113.

Bird, D.M., Kaloshian, I., 2003. Are roots special? Nematodes have their say. Physiological and Molecular Plant Pathology 62, 115–123.

Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2009. Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil. European Journal of Soil Science 60, 186–197.

Bonkowski, M., Griffiths, B., Scrimgeour, C., 2000. Substrate heterogeneity and microfauna in soil organic 'hotspots' as determinants of nitrogen capture and growth of ryegrass. Applied Soil Ecology 2000, 37–53.

Chaudhuri, S., Chatterjee, S., Katz, N., Nelson, M., Goldbaum, M., 1989. Detection of blood vessels in retinal images using two-dimensional matched filters. IEEE Transactions on Medical Imaging 8, 263–269.

Clarke, A.J., Hennessy, J., 1987. Hatching agents as stimulants of movement of Globodera rostochiensis juveniles. Revue de Nematologie 10, 471–476.

Clarkson, D.T., 1991. Root structure and sites of ion uptake. In: Waisel, Y., Eschel, A., Kalkafi, U. (Eds.), Plant Roots: the Hidden Half. Marcel Dekker, New York, pp. 417–453.

Denton, C.S., Bardgett, R.D., Cook, R., Hobbs, P.J., 1998. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biology and Biochemistry 31, 155–165.

Evans, K., Stone, A.R., 1977. A review of the distribution and biology of the potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*. PANS 23, 178–189.

Ferris, H., Bongers, T., De Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology 18, 13–29.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.

Ge, T., Wei, X., Razavi, B.S., Zhu, Z., Hu, Y., Kuzyakov, Y., Jones, D.L., Wu, J., 2017. Stability and dynamics of enzyme activity patterns in the rice rhizosphere: effects of plant growth and temperature. Soil Biology and Biochemistry 113, 108–115.

German, D.P., Chacon, S.S., Allison, S.D., 2011. Substrate concentration and enzyme allocation can affect rates of microbial decomposition. Ecology 92, 1471–1480.

Gilbert, G.A., Knight, J.D., Vance, C.P., Allan, D.L., 1999. Acid phosphatase activity in phosphorus-deficient white lupin roots. Plant, Cell and Environment 22, 801–810.

Grierson, P.F., Adams, M.A., 2000. Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (*Eucalyptus marginata* Donn ex Sm.) forest in southwestern Australia. Soil Biology and Biochemistry 32, 1817–1827.

Haase, S., Ruess, L., Neumann, G., Marhan, S., Kandeler, E., 2007. Low-level herbivory by root-knot nematodes *(Meloidogyne incognita)* modifies root hair morphology and rhizodeposition in host plants (*Hordeum vulgare*). Plant and Soil 301, 151–164.

Hallmann, J., Quadt-Hallmann, A., Miller, W.G., Sikora, R.A., Lindow, S.E., 2001. Endophytic colonization of plants by the biocontrol agent *Rhizobium etli* G12 in relation to *Meloidogyne incognita* infection. Phytopathology 91, 415–422.

Hirsch, P.R., Miller, A.J., Dennis, P.G., 2013. Do root exudates exert more influence on rhizosphere bacterial community structure than other rhizodeposition? In: de Bruijn, F.J. (Ed.), Molecular Microbial Ecology of the Rhizosphere. John Wiley & Sons, Inc.

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., Tang, X., Zhang, F., 2011. P for two, sharing a scarce resource: soil phosphorus acquisition in the rhizosphere of intercropped species. Plant Physiology 156, 1078–1086.

Hoang, T.T.D., Razavi, B.S., Blagodatskaya, E., Kuzyakov, Y., 2016. Earthworm burrows: kinetics and spatial distribution of enzymes of C-, N- and P-cycles. Soil Biology and Biochemistry 99, 94–103.

Karczmarek, A., Overmars, H., Helder, J., Goverse, A., 2004. Short communication: feeding cell development by cyst and root-knot nematodes involves a similar early, local and transient activation of a specific auxin-inducible promoter element. Molecular Plant Pathology 5, 343–346.

Klinger, J., 1965. On the orientation of plant parasitic nematodes and of some other soil animals. Nematologica 11, 4–18.

Koch, O., Tscherko, D., Kandeler, E., 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Global Biogeochemical Cycles 21, 1–11.

Kramer, S., Marhan, S., Haslwimmer, H., Ruess, L., Kandeler, E., 2013. Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. Soil Biology and Biochemistry 61, 76–85.

Liu, S., Razavi, B.S., Su, X., Maharjan, M., Zarebanadkouki, M., Blagodatskaya, E., Kuzyakov, Y., 2017. Spatio-temporal patterns of enzyme activities after manure application reflect mechanisms of niche differentiation between plants and microorganisms. Soil Biology and Biochemistry 112, 100–109.

Lynch, J., 1995. Root architecture and plant productivity. Plant Physiology 109, 7–13.

Ma, X., Razavi, B.S., Holz, M., Blagodatskaya, E., Kuzyakov, Y., 2017. Warming increases hotspot areas of enzyme activity and shortens the duration of hot moments in the root-detritusphere. Soil Biology and Biochemistry 107,

226–233.
Mathesius, U., 2003. Conservation and divergence of signaling pathways between roots and soil microbes – the Rhizobium-legume symbiosis compared to the development of lateral roots, mycorrhizal interactions and nematode-induced galls. Plant and Soil 255, 105–119.
Marinari, S., Moscatelli, C., Grego, S., 2014. Enzymes at plant-soil interface. In: Gianfreda, L., Rao, M.A. (Eds.), Enzymes in Agricultural Sciences. OMICS Group eBooks, USA, pp. 94–109.
Marschner, P., Neumann, G., Kania, A., Weiskopf, L., Lieberei, R., 2002. Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (Lupinus albus L.). Plant and Soil 246, 167–174.
Melakeberhan, H., Ferris, H., 1989. Impact of *Meloidogyne incognita* on physiological efficiency of *Vitis vinifera*. The Journal of Nematology 21, 74–80.
Miller, S.S., Liu, J., Allan, D.L., Menzhuber, C.J., Fedorova, M., Vance, C.P., 2001. Molecular control of acid phosphatase secretion into the rhizosphere of proteoid roots from phosphorus-stressed white lupin. American Society of Plant Physiologists 127, 594–606.
Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Bünemann, E., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action. Biological Processes in Soil Phosphorus Cycling. Springer, Heidelberg, pp. 215–243.
Neumann, G., Massonneau, A., Martinoia, E., Romheld, V., 1999. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. Planta 208, 373–382.
Neumann, G., Römheld, V., 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant and Soil 211, 121–130.
Neumann, G., Massonneau, A., Langlade, N., Dinkelaker, B., Hengeller, C., Römheld, V., Martinoia, E., 2000. Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (Lupinus albus L.). Annals of Botany 85, 909–919.
Neumann, G., Martinoia, E., 2002. Review: cluster roots – an underground adaptation for survival in extreme environments. Trends in Plant Science 7, 162–167.
Oteifa, B.A., 1952. Potassium nutrition of the host in relation to infection by a root-knot nematode Meloidogyne incognita. Proceedings of the Helminthological Society of Washington 19, 99–104.
Pausch, J., Tian, J., Riederer, M., Kuzyakov, Y., 2013. Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study. Plant Soil 364, 273–285.
Poll, J., Marhan, S., Hallmann, J., Kandeler, E., Ruess, L., 2007. Low amounts of root herbivory affect microbial community dynamics and carbon allocation in the rhizosphere. FEMS Microbiology Ecology 62, 268–279.
Razavi, B.S., Zarebanadkouki, M., Blagodatskaya, E., Kuzyakov, Y., 2016. Rhizosphere shape of lentil and maize: spatial distribution of enzyme activities. Soil Biology and Biochemistry 96, 229–237.
Sanaullah, M., Razavi, B.S., Blagodatskaya, E.V., Kuzyakov, Y., 2016. Spatial distribution and catalytic mechanisms of β-glucosidase activity at the root-soil interface. Biology and Fertility of Soils 52, 505–514.
Seinhorst, J.W., 1961. Plant-nematode inter-relationships. Annual Review of Microbiology 15, 177–196.
Shane, M.W., Lambers, H., 2005. Cluster roots: a curiosity in context. Plant and Soil 274, 101–125.
Sinsabaugh, R.L., Moorhead, D.L., 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26, 1305–1311.
Tarafdar, J.C., Claassen, N., 1988. Organic phosphorus compounds as a phosphorus source for higher plant through the activity of phosphatases produced by plant roots and microorganisms. Biology and Fertility of Soils 5, 308–312.
Taylor, A.L., Sasser, J.N., 1978. Biology, Identification and Control of Root-knot Nematodes (Meloidogyne Species). A cooperative publication of the Department of Plant Pathology, North Carolina State University, and the U.S. Agency for International Development. North Carolina State University Graphics, Raleigh, NC.
Van Gundy, S.D., Kirkpatrick, J.D., Golden, J., 1977. The nature and role of metabolic leakage from root-knot nematode galls and infection by *Rhizoctonia solani*. Journal of Nematology 9, 113–121.
Venkatesan, M., Gaur, H.S., Datta, S.P., 2013. Effect of root-knot nematode, *Meloidogyne graminicola* on the uptake of macronutrients and arsenic and plant growth of rice. Vegetos - An International Journal of Plant Research 26, 112–120.
Wallace, H.R., 1974. The influence of root knot nematode, *Meloidogyne Javanica*, on photosynthesis and on nutrient demand by roots of tomato plants. Nematologica 20, 27–33.
Wang, E.L.H., Bergeson, G.B., 1974. Biochemical changes in root exudate and xylem sap of tomato plants infected with *Meloigoyne incognita*. Journal of Nematology 6, 194–202.
Widdowson, J.P., Yeates, G.W., Healy, W.B., 1972. The effect of root nematodes on the utilization of phosphorus by white clover on a yellow-brown loam. New Zealand Journal of Agricultural Research 16, 77–80.
Williamson, V.M., Hussey, R.S., 1996. Nematode pathogenesis and resistance in plants. The Plant Cell 8, 1735–1745.
Williamson, V.M., Gleason, C.A., 2003. Plant-nematode interactions. Plant Biology 6, 327–333.
Yeates, G.W., Saggar, S., Denton, C.S., Mercer, C.F., 1998. Impact of clover cyst nematode (Heterodera trifolii) infection on soil microbial activity in the rhizosphere of white clover (Trifolium repens)-a pulse-labelling experiment. Nematologica 44, 81–90.
Yeates, G.W., Saggar, S., Hedley, C.B., Mercer, C.F., 1999. Increase in ^{14}C-carbon translocation to the soil microbial biomass when five species of plant-parasitic nematodes infect roots of white clover. Nematology 1, 295–300.