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Rhizodeposition and biotic interactions in the rhizosphere of *Phaseolus vulgaris* L. and *Hordeum vulgare* L.

Dissertation

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Stuttgart, den 11.01.2008 Susan Haase

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1 Summary

Biochemical processes at the soil-plant interface are largely regulated by organic and inorganic compounds released by roots and microorganisms. Several abiotic and biotic factors are suspected to stimulate rhizodeposition and, thus, contribute to enriching of the rhizosphere with plant-derived compounds. This thesis focused on the effects of two factors, (i) the elevation of atmospheric CO_2 concentration accompanied by nutrient limitation in the soil and (ii) low-level root infestation by plant-parasitic nematodes, on the quantity and quality of rhizodeposits with consequences for plant-nutrient acquisition and plant-microbial interactions in the rhizosphere. Experiments were largely conducted in mini-rhizotrones, which allowed a localized collection of rhizodeposits and rhizosphere soil along single roots.

Since the beginning of the industrial revolution atmospheric CO_2 concentrations have been steadily increasing. This probably impacts terrestrial ecosystems by stimulating plant photosynthesis and belowground allocation of the additional fixed C. Increased root exudation, promoting rhizosphere microbes, has been hypothesized as a possible explanation for the lower plant N nutritional status under elevated CO_2, due to enhanced plant-microbial N competition. Legumes may counterbalance the enhanced N requirement by increased symbiotic N_2 fixation. The effects of elevated CO_2 on factors determining this symbiotic interaction were assessed in *Phaseolus vulgaris* L. grown under limited or sufficient N supply and ambient or elevated CO_2 concentration. Elevated CO_2 reduced N tissue concentrations but did not affect plant biomass production. $^{14}CO_2$ pulse-labelling revealed no indication for a general increase in root exudation by the whole root system, which might have forced N-competition in the rhizosphere under elevated CO_2. However, a CO_2-induced stimulation in the exudation of sugars and malate, a chemoattractant for rhizobia, was detected in apical root zones, as potential infection sites. In nodules, elevated CO_2 increased the accumulation of malate as a major C source for the microsymbiont and of malonate, with functions in nodule development. Nodule biomass was also enhanced. Moreover, the release of *nod*-gene-inducing flavonoids was stimulated under elevated CO_2, suggesting a selective stimulation of factors involved in establishing the *Rhizobium* symbiosis.

Since elevated-CO_2-mediated effects on exudation by *Phaseolus vulgaris* L. are restricted to root apices, the abundance and function of the soil microbial community were investigated at two levels of spatial resolution to assess the response of microorganisms in the rhizosphere of the whole root system and in apical root zones to elevated CO_2 and different N supply. At the coarser resolution, the microbial community did not respond to CO_2 elevation because the C flux from the whole root system into soil did not change. At the higher spatial resolution, the

CO_2-mediated enhanced root exudation from root apices led to higher enzyme activities of the C and N cycle in the adhering soil at an early stage of plant growth. At later stages, however, enzyme activities decreased under elevated CO_2. This might reflect a shift in microbial C usage from the decay of polymers towards soluble carbohydrates derived from increased root exudation. CO_2 elevation or N supply did not affect the abundance of total and denitrifying bacteria in rhizosphere soil of apical root zones. Thus, the microbial community in the rhizosphere of bean plants responded to elevated CO_2 by altered enzyme regulation and not by enhanced growth.

Beyond N, plants and microorganisms may also compete for micronutrients such as Fe in the rhizosphere. *Hordeum vulgare* L., a model plant with high secretion of phytosiderophores (PS) under Fe limitation, was investigated to assess the effects of elevated CO_2 on PS release, Fe acquisition and potential impacts on rhizosphere microbial communities. Experiments were conducted in hydroponics and soil culture with or without Fe-fertilization and ambient or elevated CO_2 concentration. Elevated CO_2 stimulated biomass production of Fe-sufficient and Fe-deficient plants in both culture systems. Secretion of PS in apical root zones of N deficient plants increased strongly under elevated CO_2 in hydroponics, but no PS were detectable in root exudates from soil-grown plants. However, higher Fe shoot-contents of plants grown in soil culture without Fe supply suggest an increased efficiency for Fe acquisition under elevated CO_2. Despite the evidence for altered PS secretion under elevated CO_2, no significant influence on rhizosphere-bacterial communities was detected.

Low-level herbivory by parasitic nematodes is thought to induce leakage of plant metabolites from damaged roots, which can foster microorganisms. Other factors such as alterations in root exudation or morphology in undamaged roots, caused by nematode-host interactions were almost not considered yet. *Hordeum vulgare* L. was inoculated with 0, 2000, 4000 or 8000 root-knot nematodes (*Meloidogyne incognita)* for 4 weeks. In treatments with 4000 nematodes, shoot biomass, total N and P content increased by the end of the experiment. One week after inoculation, greater release of sugars, carboxylates and amino acids from apical root zones indicates leakage from this main nematode penetration site. Low levels of root herbivory stimulated root hair elongation in both infected and uninfected roots. This probably contributed to the increased sugar exudation in uninfected roots in all nematode treatments at three weeks after inoculation. Root-knots formed a separate microhabitat within the root system. They were characterised by decreased rhizodeposition and an increased fungal to bacterial ratio in the surrounding soil. This study provides evidence that, beside leakage, low-level root herbivory induces local and systemic effects on root morphology and exudation, which in turn may affect plant performance and competition.

In conclusion, this thesis extends our knowledge about the potential impact of two different plant-growth-affecting factors on rhizosphere processes, particularly at the small scale and is, thus, interesting for future assessment of management strategies in agriculture under global climate change.

2 Zusammenfassung

Biochemische Prozesse in der Rhizosphäre werden in hohem Maße durch organische und anorganische Verbindungen, welche passiv und/oder aktiv von Pflanzenwurzeln abgegeben werden, gesteuert. Dieser als Rhizodeposition bekannte Prozess kann durch verschiedene abiotische und biotische Faktoren stimuliert werden, was eine Anreicherung der Rhizosphäre mit leicht verfügbaren, pflanzenbürtigen Verbindungen zur Folge hat. Die vorliegende Arbeit beschäftigt sich mit den Auswirkungen der Faktoren (i) erhöhte atmosphärische CO_2-Konzentration gekoppelt mit einer Limitierung verschiedener Nährstoffe im Boden und (ii) geringer Befall durch pflanzen-parasitische Nematoden auf die Menge und Zusammensetzung der Rhizodeposition. Weiterhin werden mögliche Effekte auf die pflanzliche Nährstoffaneignung und die Interaktionen zwischen Pflanze und Mirkoorganismen in der Rhizosphäre näher beleuchtet. Die Arbeit umfasst vier Fall-Studien in denen Pflanzen in Wurzelkästen (Mini-Rhizotronen) kultiviert wurden. Die Verwendung dieser Kultivierungssysteme ermöglicht eine lokalisierte Sammlung von Rhizodepositen und Rhizosphärenboden entlang einzelner Wurzeln.

Seit Beginn der „Industriellen Revolution“ sind die CO_2-Konzentrationen in der Atmosphäre durch menschliche Aktivitäten stetig angestiegen, was auch Auswirkungen auf terrestrische Ökosysteme haben kann. Diese umfassen unter anderem eine Stimulierung der Photosynthese und eine verstärkte Verlagerung des zusätzlich fixierten C in die Wurzeln, was zu erhöhter Wurzelexsudation führen kann. Eine verstärkte Exsudation kann mikrobielles Wachstum in der Rhizosphäre fördern und damit die Konkurrenz um Nährstoffe wie N zwischen Pflanzen und Mikroorganismen verstärken. Dies stellt eine mögliche Erklärung für den schon in früheren Studien beobachteten, geringeren N-Ernährungsstatus von Pflanzen unter erhöhten CO_2-Konzentrationen dar. Leguminosen sind in der Lage, diesen erhöhten N-Bedarf durch gesteigerte, symbiotische N_2-Fixierung unter „hoch-CO_2“ auszugleichen. Es ist jedoch bisher unbekannt, inwieweit sich eine „CO_2-Erhöhung“ auf Faktoren, welche zur Stimulierung dieser Symbiose beitragen können, auswirkt. In der ersten Studie wurden daher die Effekte von erhöhter CO_2-Konzentration auf mögliche Einflussfaktoren untersucht. Als Modellpflanze wurde Bohne (*Phaseolus vulgaris* L.) ausgewählt, da sie ein gut charakterisiertes Muster von Wurzelexsudaten (Flavonoide und Carboxylate), welche Signalverbindungen bei der Induzierung der symbiotischen N_2-Fixierung sind, aufweist. Die Pflanzen wurden unter zwei N-Düngungsniveaus (limitierende und ausreichende N-Zugabe) sowie unter ambienter (400 µmol mol^{-1}) oder erhöhter (800 µmol mol^{-1}) CO_2-Konzentration kultiviert. „Hoch-CO_2“ führte zu reduzierten N Spross- und Wurzelkonzentrationen, hatte aber keinen Effekt auf die pflanzliche Biomasseproduktion. Die Ergebnisse eines $^{14}CO_2$-„Tracer“-Markierungsexperimentes zeigten keinen generellen Anstieg der Wurzelexsudation von der Gesamtwurzel unter erhöhter CO_2-Konzentration, welcher zu einer verstärkten Nährstoffkonkurrenz zwischen Pflanzen und Mikroorgansimen in der Rhizosphäre hätte führen können. Jedoch stimulierte „hoch-CO_2“ die Exsudation von Zucker und Malat, einer Locksubstanz für Rhizobien, in der apikalen Wurzelzone, den potenziellen Infektionsort für Rhizobien. Im Knöllchen-Gewebe waren unter „erhöhtem CO_2“ die Konzentrationen von Malat, die Haupt-C-Quelle des Mikro-Symbionten, und Malonat, was Funktionen bei der Knöllchenentwicklung hat, erhöht. Entsprechend war auch die Anzahl und Biomasse der Knöllchen erhöht. Darüber hinaus führte „hoch-CO_2“ zu einer verstärkten Exsudation von *nod*-Gen induzierenden Flavonoiden, was ebenfalls auf eine selektive Stimulierung der Rhizobien-Symbiose hinweist.

Aufgrund der Beobachtung aus der ersten Studie, dass sich die „CO_2-Effekte“ auf die Wurzelexsudation bei Bohne auf den apikalen Wurzelraum beschränken, wurde in der zweiten Studie die Abundanz und Funktion der mikrobiellen Rhizosphärengemeinschaft auf zwei unterschiedlichen, räumlichen Ebenen untersucht. Dazu wurden die Reaktionen der Mikroorganismen auf erhöhte CO_2-Konzentration und zwei N-Düngungsstufen in der Rhizosphäre des gesamten Wurzelraumes sowie im Bereich der apikalen Wurzelzone untersucht. Die erhöhte CO_2-Konzentration hatte keinen Einfluss auf die mikrobielle Gemeinschaft in der Rhizosphäre des gesamten Wurzelraumes, was auf die unveränderte C-Exsudation der Gesamtwurzel zurückzuführen ist. Die durch „CO_2-Erhöhung“ verstärkte Exsudation in der apikalen Wurzelzone führte aber zu höheren Enzymaktivitäten des C- und N-Kreislaufes im apikalen Rhizosphärenboden. Jedoch waren zu einem späteren Entwicklungsstadium der Pflanzen diese Enzymaktivitäten unter „hoch-CO_2“ erniedrigt. Dies könnte eine Verschiebung der mikrobiellen C Nutzung aus dem Abbau von Polymeren hin zur vermehrten mikrobiellen Aufnahme von leicht verfügbaren Kohlenhydraten aus verstärkter Wurzelexsudation widerspiegeln. Weder „CO_2-Erhöhung“ noch N-Zugabe hatten einen Effekt auf die Abundanz der gesamten sowie der denitrifizierenden Bakteriengemeinschaft im apikalen Rhizosphärenboden. Es lässt sich daher festhalten, dass die mikrobielle Gemeinschaft in der Rhizosphäre auf eine Erhöhung der CO_2-Konzentration mit einer Regulation von Enzymen (Produktion und Aktivität) und nicht mit verstärktem Wachstum reagierte.

Neben N, können Pflanzen und Mikroorganismen in der Rhizosphäre auch um Mikro-Nährstoffe wie Fe konkurrieren. In der dritten Studie wurde Gerste (*Hordeum vulgare* L.) als Modellpflanze, welche unter Fe-Mangel verstärkt Fe mobilisierende Phytosiderophore (PS) abgibt, ausgewählt. Ziel war es, die Auswirkungen einer erhöhten CO_2-Konzentration auf die PS-Abgabe, die pflanzliche Fe-Aneignung und mögliche Effekte auf die mikrobielle

Rhizosphärengemeinschaft zu untersuchen. Dazu wurde Gerste mit oder ohne Fe-Düngung sowie unter ambienter (400 µmol mol^{-1}) oder erhöhter (800 µmol mol^{-1}) CO_2-Konzentration in Nährlösung oder kalkhaltigem Boden kultiviert. In beiden Kultivierungssystemen stimulierte „hoch-CO_2“ die Biomasseproduktion sowohl von Pflanzen, die ausreichend mit Fe versorgt waren, als auch von Fe-Mangel Pflanzen. Außerdem wurde beobachtet, dass „erhöhtes CO_2“ zu einem starken Anstieg der PS-Abgabe in der apikalen Wurzelzone der Fe-Mangel Pflanzen aus hydroponischer Kultur führt. Es konnte aber keine PS-Abgabe der im Boden gewachsenen Pflanzen nachgewiesen werden. Jedoch zeigten die Fe-Mangel Pflanzen aus Bodenkultur höhere Fe-Gehalte im Sprossgewebe, was auf eine erhöhte Effizienz der pflanzlichen Fe-Aneignung unter „hoch-CO_2“ hinweist. Trotz der deutlichen Anzeichen für eine veränderte PS-Exsudation unter erhöhter CO_2-Konzentration, konnte kein Effekt auf die bakterielle Rhizosphären-Gemeinschaft gefunden werden.

Die vierte Studie beschäftigt sich mit Herbivorie von pflanzenparasitischen Nematoden bei niedrigen Befallsraten. Frühere Studien haben gezeigt, dass das beim Eindringen der Nematoden ins Wurzelgewebe auftretende „Leakage“ pflanzenbürtiger Substanzen indirekte Auswirkungen auf die mikrobielle Gemeinschaft in der Rhizosphäre haben kann. Ob eine Interaktion zwischen Wirts-Pflanze und parasitischen Nematoden auch andere Veränderungen in Wurzelexsudation und/oder Wurzelmorphologie von nicht geschädigten Wurzeln im gleichen Wurzelsystem hervorruft, ist jedoch bisher unbekannt. In dieser Studie wurde Gerste und der „root-knot“ Nematode *Meloidogyne incognita* als Modell Pflanze-Parasit System ausgewählt. Jeweils zwei Wirtspflanzen pro Wurzelkasten wurden dabei mit 0, 2000, 4000 oder 8000 Nematoden für vier Wochen inokuliert. Durch Nematodenbehandlung (4000) wurde die Sprossbiomasse und die Spross N- und P-Gehalte der Pflanzen erhöht. Eine Woche nach Nematodenzugabe führte das „Leakage“ zu vermehrter Rhizodeposition von Zuckern, Carboxylaten und Aminosäuren in der apikalen Wurzelzone, welche den potenziellen Infektionsort für Nematoden darstellt. Die Wurzelhaarlänge im Bereich der Wurzelknötchen und auch der nicht infizierten apikalen Wurzelzonen waren unter Nematodenzugabe erhöht. Letzteres deutet auf einen systemischen, d.h. die ganze Wurzel betreffenden, Effekt des Nematodenbefalles hin, der nicht auf eine Befalls-bedingte Limitierung der N- oder P-Versorgung zurückzuführen war. Ein ähnlicher systemischer Effekt war auch drei Wochen nach Nematodenzugabe im Vergleich von nicht infizierten apikalen Wurzelzonen zu äquivalenten Wurzelzonen bei Kontrollpflanzen über eine erhöhte Zuckerexsudation nachweisbar. Die Wurzelknötchen bildeten im Gesamtwurzelsystem ein eigenes Mikro-Habitat. Es wurde beobachtet, dass die Rhizodeposition der Wurzelknötchen stark reduziert war und im angrenzenden Rhizosphärenboden das Pilz zu Bakterien Verhältnis sehr stark anstieg. Diese Studie hat gezeigt, dass ein niedriger Befall der Pflanze durch „root-knot“

Nematoden, neben “Leakage“, auch lokale und systemische Effekte auf Wurzelmorphologie und Exsudation hervorruft, was sich auf die Biomasseproduktion und Konkurrenzfähigkeit der Wirtspflanze auswirken kann.

Zusammenfassend lässt sich festhalten, dass die vorliegende Arbeit das Verständnis über mögliche Auswirkungen von Pflanzenwachstum beeinflussenden Faktoren auf wurzelinduzierte Mechanismen in der Rhizosphäre vertieft hat. Dieses Wissen kann zur Verbesserung von Management Strategien in der Landwirtschaft beitragen vor allem im Hinblick einer „Globalen Klimaveränderung“.

3 General Introduction

3.1 Release of organic compounds by living plant roots

Apart from their functions as organs for water and nutrient uptake as well as for anchoring the plant in soil, plant roots release a wide range of organic and inorganic compounds into their surrounding soil environment, the rhizosphere (Neumann and Römheld, 2007). In annual plant species, 20 - 60% of the photosynthetically fixed C is translocated to the roots, and a considerable proportion of this C (up to 70%) can be released into the rhizosphere. This process is termed rhizodeposition (Liljeroth, et al., 1994; Kuzyakov and Domanski, 2000). Some of this C input is rapidly metabolized by microorganisms colonising the rhizosphere (Kuzyakov, 2002). Thus, the loss of C from root epidermal and cortical cells may affect the proliferation of microorganisms within (endorhizosphere), on the surface (rhizoplane) and outside the root (ectorhizosphere) (Lynch and Whipps, 1990). Microbial concentrations in the rhizosphere can reach between 10^{10} and 10^{12} cells per gram soil, and invertebrate numbers in the rhizosphere are at least two orders of magnitude greater than in the surrounding soil (Lussenhop and Vogel, 1991, Brimecombe et al., 2007). Organic rhizodeposition comprises lysates, liberated by autolysis of sloughed-off cells and tissue, intact root border cells, as well as root exudates released passively (diffusates) or actively (secretions) from intact root cells (Jones et al., 2004; Neumann and Römheld, 2007). The amount of C released via root exudation ranged from 0.2 - 7% of the root dry matter per day (mean exudation value of 150 µg C mg^{-1} DM of root growth) (Nguyen, 2003). Although root exudation probably represents a minor C transfer pathway compared to other fluxes, its biochemical nature may be more important for ecosystem functioning than its volume (Cardon, 1996). The composition of exuded compounds demonstrates a wide variety: simple and complex sugars, amino acids, organic acids, phenolics, alcohols, polypeptides and proteins, hormones and enzymes (Nguyen, 2003). The release of major low-molecular-weight organic constituents of root exudates such as sugars, amino acids, carboxylic acids and phenolics is a passive process along the steep concentration gradient, which usually exists between the cytoplasm and the external soil solution (mM vs. µM, respectively) (Neumann and Römheld, 2007). Besides passive diffusion, plants can respond to environmental conditions by altering their excretion of organic compounds. For example, in response to nutritional deficiency stress (e.g. P or Fe deficiency) or Al toxicity in some plant species, anion channel proteins, embedded in the plasmalemma significantly increase the passive efflux of specific carboxylates (e.g. citrate, malate, oxalate) involved in mobilizing mineral nutrients and in rhizosphere detoxification (Zhang et al., 2001; Sasaki et al., 2004; Neumann and Römheld, 2007). Together with vesicle transport, anion channels have also been implicated in the release of Fe- and Zn-mobilizing phytosiderophores (nonproteinaceous amino acids), which are involved in the micronutrient acquisition of graminaceous plant species (Nishizawa and Mori, 1987; Sakaguchi et al., 1999). Moreover, plant roots can release a wide range of secondary metabolites such as flavonoids, which are known to play a multifunctional role in rhizospheric plant-microbe and plant-plant communications (Shaw et al., 2006). Most familiar is their function as a signal in initiating the highly specific legume-rhizobia symbiosis (Schultze and Kondorosi, 1998). In nano- to micromolar concentration, flavonoids bind to the Nod D receptor in the rhizobia-cell surface, triggering the induction of all other *nod* genes involved in producing the nodulation factors (Nod factors or lipochitooligosaccharides). Flavonoids are a diverse class of polyphenolic compounds that are produced as a result of plant secondary metabolism (Werner, 2007). Their release from roots is presumably regulated by vesicle transport and plasma membrane transporters (Walker, 2003).

Both the quality and quantity of root exudates vary considerably according to the plant species and the physiological status of the plant (Nguyen, 2003). Root exudation was observed to be highest in the phase of fast vegetative growth (Sauerbeck and Johnen, 1976). Exudation takes place along the root surface with longitudinal gradients, commonly with higher rates behind the root apices (Schilling et al., 1998; Darwent et al., 2003). This may also induce gradients in microbial degradation of root exudates (Marschner, 1995). The greater exudation at apical root zones is consistent with the concentration gradients of sugars and amino acids inside the root and with the diffusion of sugars through the apoplast from the phloem to the apical meristems (Jones, 1998, 1999). Moreover, intense root exudation, restricted to distinct root zones, may enhance the mobilization efficiency due to localized accumulation of exudate compounds in the rhizosphere. This effect may be further increased by a high capacity for nutrient uptake in apical root zones (Neumann and Römheld, 2007).

A range of multiple environmental factors influences the amount and composition of rhizodeposits. Since microbial composition and activity as well as species richness at the soil-plant interface are related either directly or indirectly to rhizodeposits, they may vary according to the same environmental factors that affect exudation. The studies presented in this thesis focus on the impact of elevated atmospheric CO_2 concentration accompanied by different nutrient status in soil and belowground herbivory by nematodes as factors influencing rhizosphere processes.

3.2 Elevated atmospheric CO_2 concentration

Since 1750, anthropogenic activities, particularly burning of fossil fuels (contribution 78%) and land use changes (deforestation: 22%), have led to a marked increase (35%) in the atmospheric CO_2 concentration, reaching 379 µmol mol^{-1} in 2005 (IPCC, 2007). The radiative forcing of

CO_2 and other greenhouse gases like methane and nitrous oxide has contributed to a 0.74°C temperature rise of the global surface since the late 19th century. Estimates of atmospheric CO_2 concentrations in the year 2100 range between 730 and 1088 µmol mol^{-1} depending on economic growth, technological advances and C sequestration by biological and geological processes (IPCC, 2007). The terrestrial C cycling is probably already changing in response to these perturbations, but substantial uncertainties remain about the sensitivity of ecosystems to climate-change-forcing factors (Pendall et al., 2004a). The capacity of ecosystems to store C depends on net ecosystem production, which is the balance between net primary production and heterotrophic respiration. Increasing atmospheric CO_2 concentrations enhances plant C assimilation because, particularly in C_3 plants, the key enzyme of C assimilation, ribulose-1,5-biphophate-carboxylase-oxygenase (RuBisCO), is unsaturated under ambient CO_2 conditions (Amthor et al., 2001). Thus, the rising CO_2 levels will have a major impact on single species and whole ecosystems (Cramer et al., 2001). Numerous studies demonstrated a stimulation of growth and biomass production of plants exposed to elevated CO_2 (Kimball et al., 2002; Nowak et al., 2004; Ainsworth and Long, 2005). The magnitude of the response, however, varied according to species, growing season and experimental conditions. Nutrient availability is an important factor influencing the extent of the plant response to CO_2 elevation. With increased plant growth, elevated CO_2 induces a stronger N-sink strength in plants (Zanetti et al., 1998). A reduced plant N concentration was observed for many C_3 species under elevated CO_2. This was accompanied by an increased N-use efficiency, which was explained by an acclimation of photosynthesis to elevated CO_2 along with a decrease in RuBisCO and photorespiration enzymes (Webber et al., 1994; Ainsworth and Long, 2005). In C_3 plants about 80% of total leaf N is located in the chloroplasts, with most being bound in the RuBisCO-enzyme protein (Evans, 1989). Otherwise, a reduced plant N concentration under elevated CO_2 was evoked indirectly by a reduction of plant-available N in soils, reflecting intensified competition for resources between plants and soil microbes (Diaz et al., 1993). Since symbiotic N fixation could counterbalance the CO_2-induced limitation of N availability, leguminous plants become favoured and may play a key role in the ecosystem response to elevated CO_2 (Marilley et al., 1999). The N-fixing ability of legumes is generally enhanced under elevated CO_2 (Lüscher et al., 2000) by two general mechanisms: formation of more nodules or higher specific N_2 fixation (Zanetti et al., 1998; Schortemeyer et al., 2002). Accordingly, the relative contribution of symbiotically fixed N_2 to total plant N increases under elevated CO_2 (Zanetti et al., 1996; Feng et al., 2004).

3.2.1 Effects of elevated CO_2 on belowground processes - rhizodeposition

Below-ground processes play important roles in the global C cycle because they regulate the storage of large quantities of soil organic carbon (SOC), and are potentially very sensitive to indirect effects of elevated CO_2. Numerous studies have shown that increases in plant aboveground biomass under elevated CO_2 are often smaller than expected from the increased C fixation (Körner, 1996). It has, therefore, been hypothesized that a great part of the extra C will be allocated belowground (van Ginkel et al., 2000; Zak et al., 2000). The putative consequences for rhizodeposition, which potentially contributes to the soil C pool, are still not clear. Several studies demonstrate either an increased SOC content (Williams et al., 2000) or no influence of elevated CO_2 on SOC stocks even after long-term CO_2 enrichment (Niklaus et al., 2000; Van Kessel et al., 2000; Gill et al., 2002). In those studies, the contribution of plant-derived C (i.e. C from litter fall, rhizodeposition or root turnover) to the SOC pool was assessed collectively; thus individual fluxes were not determined. Only a few studies have investigated rhizodeposition or more specific root exudation in plant-soil systems exposed to elevated CO_2, presumably due to methodological difficulties involved in quantification (Todorovic et al., 2001). With respect to total C flux from plant roots into soil, controversial results have been reported. Elevated CO_2 stimulated rhizodeposition in a native C3-C4 grassland ecosystem (Pendall et al., 2004b). Similarly, in a $^{14}CO_2$ pulse-chase labelling experiment with *Lolium perenne* grown in pots with soil from the New Zealand FACE (Free Air CO_2 Enrichment) site, more root-derived C was found in the soil 48 h after labelling, suggesting increased root exudation (Allard et al., 2006). Enhanced root exudation is a common (Cheng and Johnson, 1998), but not a general response to elevated CO_2 (Hodge and Millard, 1998; Uselman et al., 2000). $^{14}CO_2$ pulse-chase labelling of *Lolium perenne* planted into soil cores inserted within the Swiss FACE experiment revealed that C partitioning to soil was unaffected or reduced under elevated CO_2 (Bazot et al., 2006). These contrasting results probably reflect differences in the used plant species, the physiological state of the plant and experimental conditions (such as different N supply). For example, under non-limited N supply, C allocation to *Lolium perenne* roots was not affected by elevated atmospheric CO_2 (Suter et al., 2002).

An enhanced overall root exudation under elevated CO_2 can be caused either by stimulated root growth under elevated CO_2 (van Ginkel et al., 2000) or by increased exudation per unit root area (Cheng and Johnson, 1998). Qualitative alterations in the composition of root exudates towards higher C/N ratios have also been suggested under elevated CO_2 (Grayston et al., 1998). Moreover, elevation of CO_2 may improve the spatial acquisition of nutrients by stimulating fine root production (Zak et al., 2000), but may also affect the chemical availability of nutrients in the rhizosphere by increased root exudation of organic compounds, which in turn impact the mobilization of sparingly soluble nutrients. However, this aspect has so far attracted only marginal attention. In P-deficient *Lupinus albus*, elevated CO_2 treatments did not affect the amount of citrate released from individual root clusters, but citrate exudation started in earlier stages of cluster root development (Wasaki et al., 2005). This was associated with a higher number of root clusters per root system, which may indicate a CO_2-induced increase in the total release of P-mobilizing root exudates per plant. There was no effect on the respective rhizosphere concentrations. Most past studies have focused on the role of elevated atmospheric CO_2 concentration in C-, N-, and P-cycling. However, there is no information regarding CO_2 effects on the release rates of phytosiderophores, which are involved in the rhizosphere-availability of micronutrients, such as iron (Fe), zinc (Zn), or manganese (Mn) despite their function as essential mineral nutrients (Marschner, 1995), as factors determining pathogen resistance (Graham and Webb, 1991) and plant-microbial competition (Yehuda et al., 1996; Hördt et al., 2000).

3.2.2 Effects of elevated CO_2 on soil microbes

Soil microorganisms regulate the dynamics of organic matter decomposition and plant nutrient availability. They therefore play a key role in ecosystem response to global climate change. Whether soils can act as a sink or source for atmospheric CO_2 depends largely on their heterotrophic respiration of plant residues and soil organic matter (Freeman et al., 2004). Although soil microorganisms are not directly influenced by atmospheric CO_2 enrichment because the CO_2 concentrations in soil are already more than 50 times greater than in the atmosphere (van Veen et al., 1991), there may be a plant-mediated influence on microbial communities due to increased C input from rhizodeposition. The latter may enrich the rhizosphere with easily available substrates (Rogers et al., 1994). Therefore, the altered quantity and/or quality of plant-derived resources are thought to modify the activity, density and diversity of soil microbial communities. Nonetheless, the microbial responses to CO_2 elevation vary widely. In 47 published studies, changes in soil respiration (root and microbial respiration) under plants exposed to elevated CO_2 ranged from a 10% decline to a 162% increase (Zak et al., 2000). Numerous studies reported significant increases in microbial biomass, others neutral or negative responses (Zak et al., 2000; Sonneman and Wolters, 2005). The latter might be explained by limiting N availability (Diaz et al., 1993) and/or increased microbial grazing under elevated CO_2 (Yeates et al., 2003). Previous studies using methods targeting the overall microbial community structure by PLFA (phospholipid fatty acids) analysis or the eubacterial community diversity by 16S rDNA-based approaches (DGGE fingerprints, %G+C-base profiling) found no or weakly significant CO_2 effects (Griffiths et al., 1998; Hodge et al. 1998; Bruce et al., 2000; Montealegre et al., 2002; Ebersberger et al., 2004;

Wasaki et al., 2005; Drissner et al., 2007). Several authors have shown shifts in the composition of soil microbial communities, whereby those bacteria (*e.g. Pseudomonas*) colonising the rhizosphere and the rhizoplane-endorhizosphere were most affected (Schortemeyer et al., 1996; Marilley et al., 1999; Fromin et al., 2005). Moreover, elevated atmospheric CO_2 affects the competitive ability of root nodule symbionts (e.g. *Rhizobia*) in the legume rhizosphere, most likely leading to a selection of these particular strains to nodulate legumes (Montealegre et al., 2000; Marilley et al., 1999). Despite this, the factors involved in selectively stimulating the *Rhizobium* symbiosis at elevated CO_2 are not well understood. Increased root exudation of chemoattractants (malate, phenolic acids), of *nod*-gene inducing flavonoids and of other signal compounds involved in establishing the *Rhizobium* symbiosis have been discussed as possible explanations (Marilley et al., 1999).

Beyond modifying the density and structure of microbial communities, an additional input of organic substrates under elevated CO_2 may also affect microbial functions (Freeman et al., 2004). Extracellular enzyme activities, used to assess alterations in the function of soil microdecomposer communities (Marx et al., 2001), were sensitive indicators of CO_2-induced changes in different ecosystems (Kang et al., 2001; Kandeler et al., 2006b). Elevated CO_2 increased the activities of enzymes such as phosphatase and urease, which promote P or N acquisition (Ebersberger et al., 2003; Drissner et al., 2007). The activity of polysaccharide-degrading enzymes also tended to increase under elevated CO_2 (Dhillion et al., 1996; Mayr et al., 1999). This suggests changes in soil organic matter decomposition, which may alter C sequestration and plant nutrient availability (Henry et al., 2005). With increased root exudation, much of the additional C fixed under elevated CO_2 is partitioned to rapidly cycling C pools rather than into more recalcitrant fractions of soil organic matter (Hungate et al., 1997). However, the addition of labile C may induce a priming effect, fostering the breakdown of more recalcitrant SOC fractions (Cheng, 1999; Hoosbeck et al., 2004).

Understanding the effects of elevated CO_2 on the activity of rhizosphere microorganisms is crucial because changes in nutrient turnover and particularly N cycling will feedback on plant growth. Two contrasting mechanisms concerning N-availability under elevated CO_2 have been hypothesized (Hu et al., 2005): (i) Diaz et al. (1993) concluded that increased C availability under elevated CO_2 would boost microbes, in turn immobilizing nutrients and thus constraining plant responses; (ii) Zak et al. (1993) supposed a higher N-availability for plants based on increased N mineralization rates under elevated CO_2 (due to priming effects of increased rhizodeposition on the mineralization of native soil organic matter). Note, however, that enhanced as well as declined gross N mineralization and N immobilization have been reported under elevated CO_2 (Zak et al., 2000; Richter et al., 2003; Hu et al., 2005).

The effects of CO_2 on denitrification processes have attracted particular attention because these are key mechanisms returning N from terrestrial ecosystems leading to a loss of plant available N in soil, while also mediating releases of the potent greenhouse gas N_2O (Baggs, 2003). The complete denitrification pathway is a modular process comprising four sequential enzymatic reductions: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ (see review by Philippot et al., 2007). Whereas some denitrifiers can reduce NO_3^- into N_2 due to the presence of genes encoding all denitrification reductases, others have a truncated pathway (Zumft, 1997). Denitrification depends on the presence and availability of electron donors, mostly organic compounds, and of electron acceptors (nitrogen oxides, O_2). All these proximate factors could be modified by plants (exudation of organic compounds, NO_3^- assimilation) as well as by associated microbiota. Denitrifiers generally exist in soils as aerobic heterotrophs and switch to nitrate as an alternative electron acceptor only if O_2 is limited. This could be a competitive advantage for bacteria in the rhizosphere, where O_2 is limited because of root and microbial respiration (Klemedtsson et al., 1987; Højberg et al., 1996; Ghiglione et al., 2000). Moreover, an increased microbial respiration in the rhizosphere under elevated CO_2 may reduce the O_2 content and hence create anoxic sites (Zak et al., 2000). Several studies on the response of denitrification to elevated atmospheric CO_2 revealed increased rates of activity (Smart et al., 1997; Carnol, et al., 2002; Kettunen et al., 2005), boosting N_2O emission (Baggs et al., 2003). This was explained by higher availability of root-derived C and/or by higher water use efficiency of plants under elevated CO_2 (Niklaus et al., 1998), which facilitate the occurrence of anaerobic conditions (Körner, 2000). We still know little about the response of microorganisms involved in denitrification to elevated CO_2 (Deiglmayr et al., 2004; Fromin et al., 2005; Roussel-Delif et al., 2005). Roussel-Delif et al. (2005) showed that the frequency of root-associated nitrate-dissimilating *Pseudomonas* in low fertilised *Lolium perenne* swards was increased under elevated CO_2. Fromin et al. (2005) suggested that N availability may be a major factor influencing denitrifier populations under elevated CO_2, most likely due to increased competition for N between plants and bacteria. Limiting NO_3^- concentrations were also considered to be responsible for the neutral effect of elevated CO_2 on denitrifying processes (Martín-Olmedo et al., 2002; Barnard et al., 2004).

These variable responses of microbes to elevated CO_2 suggest that the complexity of the systems studied obscures a fundamental understanding of the processes involved. To date, however, no clear understanding has emerged on how root exudation (quantity and quality) is affected by elevated CO_2 concentrations with consequences for plant-nutrient acquisition and plant-microbial interactions in the rhizosphere.

3.3 Belowground herbivory by plant-parasitic nematodes

Over the last decade, the mechanisms by which plant herbivory indirectly affects soil biota and associated belowground processes like decomposition and nutrient cycling have been increasingly recognized (Bardgett et al., 1998; Van der Putten et al., 2001; Wardle et al., 2004). Aboveground herbivory can affect soil organisms and soil ecosystem-level processes significantly through the alteration of detrital inputs (e.g. plant litter) into soil but also by non-detrital inputs through changes in plant C allocation leading to altered root exudation. (Bardgett and Wardle, 2003). ^{14}C-CO_2 pulse labelling experiments have shown that above-ground herbivory enhances root exudation (Holland et al., 1996). This in turn, may promote rhizosphere microbial populations and facilitate nutrient mineralization and plant growth (Mawdsley and Bardgett , 1997; Bardgett et al., 1998; Hamilton and Frank, 2001).

Similar processes probably also occur belowground, mediated by the root-feeding fauna (Bardgett et al., 1999a; Grayston et al., 2001). Nematodes are the most abundant and, perhaps, functionally eminent faunal group in terrestrial ecosystems. For example, their densities in grassland surface soils tend to be in the region of 3 - 4 million specimens per m^{-2} (Yeates et al., 1997). Nematode communities in soil may also display tremendous taxonomic richness. Most of the nematode species can be allocated to specific feeding groups, in particular plant-feeders (28 - 53% of total nematodes) and bacterial-feeders (20 - 36% of total nematodes) (Bardgett et al., 1999a). Plant-parasitic nematodes are often referred to as "hidden enemies" (Cohn et al., 2002). Depending on their feeding mode, they break down root cell structure, remove cell matters, disrupt physiological processes and modify the genetic expression of their host plant (Manzanilla-López et al., 2003). While these primary effects of plant-parasitic nematodes have received considerable attention, indirect effects on rhizosphere processes are poorly understood. Yeates et al. (1998) used the ^{14}C-CO_2 pulse labelling technique to show that, over a 14-day period, infection of *Trifolium repens* roots by low numbers of clover cyst nematodes (*Heterodera trifolii*), at average field densities (approximately 25 nematodes per plant), slightly increased the translocation of photoassimilate ^{14}C to roots, and led to a significant increase in the leakage of ^{14}C from roots, resulting in an enhanced microbial ^{14}C content in the rhizosphere. Such infections can thus increase plant-derived C and N inputs to soil and may contribute to the regulation of microbial communities and nutrient cycling resulting in an altered availability of plant nutrients in soil. (Denton et al., 1999; Bardgett et al., 1999b; Tu et al., 2003). One suggestion is that low amounts of root infestation may reduce the overall stress on infected plants (Yeates et al., 1998; Bardgett et al., 1999a). Moreover, Dromph et al. (2006) have shown that *Heterodera trifolii* can influence vegetation characteristics by increasing the transfer of plant-derived N from their host, a N-fixing legume (*Trifolium repens*), to neighbouring grass species, thereby benefiting the growth of the neighbour.

Evidence for altered nutrient inputs to soil due to low levels of root infestation by nematodes comes mostly from grassland studies on the clover cyst nematode. Plant responses, however, may also depend on nematode feeding strategies (Yeates et al., 1999). Besides cyst nematodes including the genera *Heterodera*, the economically most important group are root-knot nematodes, particularly the genus *Meloidogyne*. The latter infect thousands of different plant species, including many crops, and may cause severe yield losses (Bird, 2004). Little, however, is known about the vigour of root-knots nematodes at low levels of root herbivory in agroecosystems. Understanding whether and how parasitic nematodes affect nutrient flow to the rhizosphere is crucial because related changes in nutrient cycling may in turn affect plant performance and competition.

3.4 Collection of root exudates

Various techniques have been used to collect root exudates. Water-soluble exudates are most frequently collected from plants grown in nutrient solutions using collection techniques with trap solution. Although these methods broadly indicate exudates patterns, assigning the results to actual soil culture conditions remains difficult. For example, mechanical impedance of solid growth media as well as soil microorganisms may affect both root morphology and the total amount and composition of substances released by the root (Boeuf-Tremblay et al., 1995; Brimecombe et al., 2007; Neumann and Römheld, 2007). Alternatively, root exudates can be collected from soil-grown plants by percolating culture vessels with trap solution. Diffusion of root-derived C into soil can also be studied with two compartment systems (root mat technique), which commonly use porous membranes to separate soil-root from rhizosphere compartments (Kuzyakov, 2006). Nonetheless, these methods can not provide information on the spatial localization of the root exudation process. In many plants, root exudation is not uniformly distributed over the whole root system (Neumann and Römheld, 2007). For example, longitudinal gradients of flavonoid exudation along the roots have been observed in soybean seedlings (Kape et al., 1992), and a considerable spatial variation has been reported for the release of phytosiderophores, which predominantly takes place in apical root zones (Marschner et al., 1986). Moreover, the apical root zone – as the site of root hair formation and root elongation - is also a potential infection site for rhizobia and plant-parasitic nematodes. Microsuction cups made of HPLC capillaries were successfully used for localized collection of compounds released into the soil solution (Göttlein et al., 1996; Sandnes et al., 2005). Another approach to study spatial variation of root exudation along single roots uses sorption media with high soaking capacity (e.g. filter paper discs made of chromatography paper, nylon membranes); these are applied onto the root surface of soil-grown plants (Neumann, 2007) (Fig. 3.1). Accordingly, plants were grown in mini-rhizotrones (rhizoboxes), equipped with removable perspex front sheets to enable access to the root surface (Fig. 3.2). The exudates can be re-extracted from the filter papers and calculation of exudation rates refers to root length (non-destructive) or biomass (destructive) of the selected root zones. The non-destructive sampling also enables repeated measurement during plant growth (Neumann, 2007). Finally, using mini-rhizotrones also enables the collection of rhizosphere soil along single roots at a small scale.

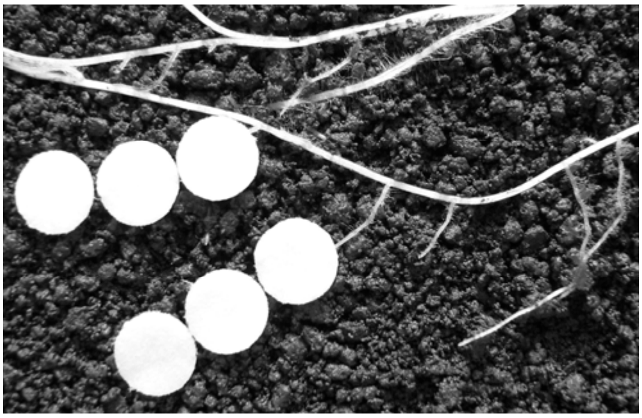

Fig. 3.1: Localized collection of root exudates from soil grown plants by use of sorption media (filter papers).

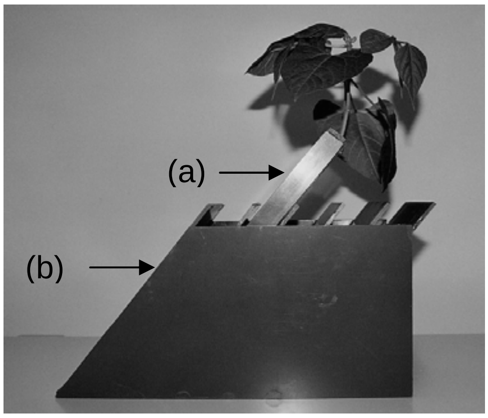

Fig. 3.2: (a) PVC mini-rhizotron (rhizobox) consisting of a corpus with irrigation holes and a perspex front lid. (b) Rhizobox holder for fixing rhizoboxes during the culture period.

A major problem of all techniques used to collect root exudates is the risk of microbial degradation during the collection period. The ability to differentiate between root exudates and microbial metabolites in the rhizosphere is also limited. Short-term collection periods (1-2 h) can minimize the risk of microbial degradation, and short-term isotopic labelling techniques (pulse-chase labelling) can help to differentiate between root exudates and microbial metabolites. Note also that only compounds dissolved in the rhizosphere solution are captured by the filter paper technique, but considerable amounts of root-derived organic compounds can be adsorbed to the soil matrix or to the cell wall. This requires recovery experiments to evaluate sorption effects to the soil matrix (Neumann, 2007). Moreover, localized sampling can also be restricted by very fine roots or low levels of soil moisture, and small sample volumes can be a limiting factor in analysing root exudates (Neumann, 2007).

4 Thesis Outline

This thesis uses four case studies to assess the effects of (i) elevated atmospheric CO_2 concentration accompanied by nutrient limitation in soil and (ii) low-level root infestation by plant parasitic nematodes on rhizodeposition and its consequences for plant-nutrient acquisition, plant-microbial interactions and nutrient cycling in the rhizosphere.

In the first study, the impact of elevated CO_2 on factors involved in establishing and maintaining the *Rhizobium* symbiosis in leguminous plants was examined. *Phaseolus vulgaris* L. was used as a fast-growing model plant with a well-characterized pattern of root exudates, flavonoids and carboxylates that function as signal compounds in establishing the symbiotic N_2 fixation. Our investigation was based on earlier studies, which revealed that many C_3 plants have a lower N nutritional status under elevated CO_2 and that leguminous plants may be able to counterbalance the enhanced N requirement by increased symbiotic N_2 fixation. We aimed to verify (i) whether elevated CO_2 limits N availability in the rhizosphere by plant-microbial competition stimulated by increased root exudation and (ii) whether in leguminous plants the lower N-nutritional status promotes symbiotic N_2 fixation by increased root exudation of signal compounds and/or improved carbon supply to the microsymbiont. Plants were grown under limited or sufficient N supply at ambient or elevated CO_2 concentrations in mini-rhizotrones. This allowed the localized collection of exudates in the apical root zone, as the potential infection site for rhizobia. Moreover, short-term $^{14}CO_2$ pulse-labelling of photoassimilates was employed to investigate the pattern of current assimilate partitioning between plant compartments and soil C pools.

As alterations in C release from plant roots are thought to influence rhizosphere microbial growth and maintenance, the second study investigated the abundance and function of the soil microbial community. The goal was to assess the response of microorganisms to elevated CO_2 and different N supply in the rhizosphere of *Phaseolus vugaris* L.. We hypothesized that specific members of the soil microbial community profit from the additional release of plant-derived C under elevated CO_2. Since the first study of this thesis revealed that elevated-CO_2-mediated effects on root exudation are restricted to apical root zones, this study was performed at two levels of spatial resolution. At the coarser resolution, we characterized the soil microbial community in the rhizosphere soil (total root adhering soil) using microbial biomass and basal respiration determination as well as the phospholipid fatty acids (PLFAs) pattern. At the higher spatial resolution (soil surrounding apical root zones), we focused on specific functions of the soil microbial community involved in C, N and P cycling. Accordingly, different extracellular soil enzymes as well as densities of both total bacteria and denitrifiers were estimated.

The third experiment focused on the role of elevated atmospheric CO_2 concentrations on the rhizosphere-availability of micronutrients such as iron (Fe). We used *Hordeum vulgare* L. as an Fe-efficient model plant with a high potential for biosynthesis and secretion of phytosiderophores (PS) under Fe deficiency stress. The goal was to assess the effects of elevated CO_2 on (i) plant Fe acquisition, (ii) root-induced changes in rhizosphere chemistry and (iii) related impacts on rhizosphere microbial communities in apical root zones, which show the highest PS release rates. Experiments were conducted in hydroponic culture and in soil culture (mini-rhizotrones) with a calcareous sub-soil under ambient and elevated atmospheric CO_2 concentrations with and without Fe fertilization. The diversity and function of rhizosphere microbial communities were characterized by DGGE analysis of 16S rRNA and by measuring extracellular enzyme activities, respectively.

The fourth study investigated the impact of plant parasitic nematodes at low infestation rates on rhizodepostion of the host plant and related effects on rhizosphere microorganisms. Our specific objectives were to examine if a low level of root herbivory by nematodes results in (i) direct effects such as leakage from damaged roots, (ii) indirect modifications in root morphology or exudation, (iii) systemic host plant responses of undamaged root parts, and (iv) changes in the microbial community structure. The root-knot nematode *Meloidogyne incognita* and *Hordeum vulgare* L. was used as a model parasite-plant system. Plants were grown in mini-rhizotrones and were infected with different densities of *M. incognita* well below the threshold level for plant damage. Root morphology, rhizodeposition and rhizosphere microbial communities (PLFA pattern) were assessed in apical root zones, the major nematode infection site. Experiments comprised a complete life cycle of *M. incognita*, and the developed root-knots were investigated as a separate microhabitat.

5 Elevation of atmospheric CO_2 and N-nutritional status modify nodulation, nodule-carbon supply, and root exudation of *Phaseolus vulgaris* L.

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5.1 Abstract

Increased root exudation and a related stimulation of rhizosphere-microbial growth have been hypothesised as possible explanations for a lower nitrogen- (N-) nutritional status of plants grown under elevated atmospheric CO_2 concentrations, due to enhanced plant-microbial N competition in the rhizosphere. Leguminous plants may be able to counterbalance the enhanced N requirement by increased symbiotic N_2 fixation. Only limited information is available about the factors determining the stimulation of symbiotic N_2 fixation in response to elevated CO_2.
In this study, short-term effects of elevated CO_2 on quality and quantity of root exudation, and on carbon supply to the nodules were assessed in *Phaseolus vulgaris,* grown in soil culture with limited (30 mg N kg^{-1} soil) and sufficient N supply (200 mg N kg^{-1} soil), at ambient (400 µmol mol^{-1}) and elevated (800 µmol mol^{-1}) atmospheric CO_2 concentrations.
Elevated CO_2 reduced N tissue concentrations in both N treatments, accelerated the expression of N deficiency symptoms in the N-limited variant, but did not affect plant biomass production. $^{14}CO_2$ pulse-chase labelling revealed no indication for a general increase in root exudation with subsequent stimulation of rhizosphere microbial growth, resulting in increased N-competition in the rhizosphere at elevated CO_2. However, a CO_2-induced stimulation in root exudation of sugars and malate as a chemo-attractant for rhizobia was detected in 0.5-1.5 cm apical root zones as potential infection sites. Particularly in nodules, elevated CO_2 increased the accumulation of malate as a major carbon source for the microsymbiont and of malonate with essential functions for nodule development. Nodule number, biomass and the proportion of leghaemoglobin-producing nodules were also enhanced. The release of *nod*-gene-inducing flavonoids (genistein, daidzein and coumestrol) was stimulated under elevated CO_2, independent of the N supply, and was already detectable at early stages of seedling development at 6 days after sowing.

6 Local response of bacterial densities and enzyme activities to elevated atmospheric CO_2 and different N supply in the rhizosphere of *Phaseolus vulgaris* L.

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6.1 Abstract

Altered flux of labile C from plant roots into soil is thought to influence growth and maintenance of microbial communities under elevated atmospheric CO_2 concentrations. We studied the abundance and function of the soil microbial community at two levels of spatial resolution to assess the response of microorganisms in the rhizosphere of the whole root system and of apical root zones of *Phaseolus vulgaris* L. to elevated CO_2 and high or low N supply.

At the coarser resolution microbial biomass C, basal respiration and phospholipid fatty acid (PLFA) patterns in the rhizosphere remained unaffected by elevated CO_2, because the C flux from the whole root system into soil did not change [as shown by Haase, S., Neumann, G., Kania, A., Kuzyakov, Y., Römheld, V., Kandeler E., 2007. Elevation of atmospheric CO_2 and N-nutritional status modify nodulation, nodule carbon supply, and root exudation of *Phaseolus vulgaris* L. Soil Biology & Biochemistry 39, 2208-2221]. At a higher spatial resolution, more low molecular weight compounds were released from apical root zones under elevated CO_2. Thus, at an early stage of plant growth (12 days after sowing), elevated CO_2 induced an increase of enzyme activities (xylosidase, cellobiosidase and leucine-aminopeptidase) in rhizosphere soil of apical root zones. At later stages of plant growth (21 days after sowing), however, enzyme activities (those above as well as N-acetyl-*ß*-glucosaminidase and phosphatase) decreased under elevated CO_2. The abundance of total and denitrifying bacteria in rhizosphere soil of apical root zones was unaffected by CO_2 elevation or N supply. Plant age seemed to be the main factor influencing the density of the bacterial community. In conclusion, the soil microbial community in the apical root zone responded to elevated CO_2 by altered enzyme regulation (production and/or activity) and not by greater bacterial abundance.

7 Responses to iron limitation in *Hordeum vulgare* L. as affected by the atmospheric CO_2 concentration

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7.1 Abstract

Hordeum vulgare was used as an iron-efficient model plant, with a high potential for biosynthesis and secretion of phytosiderophores (PS) under iron (Fe) deficiency stress, to assess effects of elevated atmospheric CO_2 concentrations on Fe acquisition, the release of PS, and potential impacts on the functional and structural diversity of rhizosphere microbial communities. Experiments were conducted in hydroponic culture and in rhizobox microcosms with a calcareous sub-soil under ambient (400 µmol mol^{-1}) and elevated (800 µmol mol^{-1}) atmospheric CO_2 concentrations with and without Fe fertilization. Elevated CO_2 treatments stimulated biomass production in Fe-sufficient and Fe-deficient plants, both in hydroponics and in soil culture. Root/shoot biomass ratio was increased in severely Fe-deficient plants grown in hydroponics but not under moderate Fe limitation in soil culture. Significantly increased biomass production in high CO_2 treatments, even under severe Fe deficiency in hydroponic culture, indicates an improved internal Fe utilization. Iron deficiency-induced secretion of PS in 0.5 - 2.5 cm sub-apical root zones was increased by 74% in response to elevated CO_2 treatments of barley plants in hydroponics but no PS were detectable in root exudates collected from soil-grown plants. This may be attributed to suppression of PS release by internal Fe concentrations above the critical level for Fe deficiency, determined at final harvest for soil grown barley plants, even without additional Fe supply. However, extremely low concentrations of easily plant-available Fe forms (Na-acetate extract: 0 mg kg^{-1}, DTPA extract: 2.5 mg kg^{-1}) in the investigated soil and low Fe seed reserves, suggest a contribution of PS-mediated Fe mobilisation from sparingly soluble Fe sources to Fe acquisition of the soil-grown barley plants during the preceeding culture period. Higher Fe contents in shoots (+52%) of plants grown in soil culture without Fe supply under elevated atmospheric CO_2 concentrations may indicate an increased efficiency for Fe acquisition. Although, there is evidence for alterations in root growth and PS secretion rates of *Hordeum vulgare* in response to Fe deficiency and elevated atmospheric CO_2 concentrations, no significant influence on diversity and function of rhizosphere-bacterial communities was detectable in the outer rhizosphere soil (0 - 3 mm distance from the root surface) by DGGE of 16S rRNA gene fragments and analysis of marker enzyme activities for C-, N-, and P-cycles.

8 Low-level herbivory by root-knot nematodes (*Meloidogyne incognita*) modifies root hair morphology and rhizodeposition in host plants (*Hordeum vulgare*)

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8.1 Abstract

Low amounts of root infestation by plant parasitic nematodes are suggested to increase nutrient supply and in turn enhance microbial activity and net mineralization rate in the rhizosphere. These effects are generally related to “leakage” of plant-derived metabolites from damaged roots. Besides leakage, the present study examines other nematode-host interactions such as alterations in root exudation and morphology, which were almost not considered yet. This includes undamaged root parts in order to assess systemic plant response. The root-knot nematode *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949) and barley (*Hordeum vulgare* L. cv. *Europa*) was used as model system. Host plants were grown in mini-rhizotrones inoculated with 0, 2000, 4000 or 8000 *M. incognita* for four weeks. Root morphology, rhizodeposition (sugars, carboxylates, amino acids), and rhizosphere microbial communities (PLFAs) were assessed. In treatments with 4000 nematodes, shoot biomass, total N and P content increased by the end of the experiment. Generally, an enhanced release of plant metabolites (sugars, carboxylates, amino acids) from the apical root zone occurred one week after inoculation with 4000 and 8000 *M. incognita*, indicating root leakage. Low levels of root herbivory stimulated root hair elongation in both infected and uninfected roots. These systemic changes in root morphology likely contributed to the increased sugar exudation in uninfected roots in all nematode treatments at three weeks after inoculation. Root-knots formed a separate microhabitat within the root-system. They were characterised by decreased rhizodeposition and increased fungal to bacterial ratio in the adhering rhizosphere soil. The present study provides the first evidence that, apart from leakage, nematode root herbivory at background levels induces local and systemic effects on root morphology and exudation, which in turn may affect plant performance.

9 General Discussion and Concluding Remarks

The present thesis investigated the effects of elevated CO_2 and low-level root herbivory on rhizodeposition, along with its consequences for plant-nutrient acquisition and plant-microbial interactions in the rhizosphere, based on two different model plants: *Phaseolus vulgaris* and *Hordeum vulgare*.

9.1 Effects of elevated atmospheric CO_2 concentration

This thesis yields valuable information about the influence of elevated atmospheric CO_2 concentration on root exudation related to nutrient acquisition by plants grown under Fe or N deficiency. Our results suggest a selective stimulation of factors involved in establishing and maintaining the *Rhizobium* symbiosis in *Phaseolus vulgaris* at elevated CO_2. This was expressed in increased exudation of signal flavonoids, elevated root exudation of chemo-attractants from apical root zones (as potential infection sites for rhizobia) and increased tissue concentration of carboxylates in nodules. In *Hordeum vulgare*, elevated atmospheric CO_2 concentrations strongly impacted the expression of adaptations to Fe deficiency. The CO_2-induced modifications comprised: increased internal Fe use efficiency, stimulated root growth and increased root exudation of Fe-mobilizing phytosiderophores from apical root zones. Both aspects – the stimulation of factors regulating symbiotic N_2 fixation and the improved efficiency of the Strategy II mechanism for acquiring micronutrients (Fe, Zn) in response to elevated CO_2 – may influence the interspecific competition in plant communities, particularly on calcareous soils with low Fe availability or on soils with low N availability. Further studies are necessary to investigate whether similar responses can also be expected under natural field conditions and in other leguminous or graminaceous plants. The case study with bean demonstrates that a CO_2-induced stimulation of photosynthesis, associated with the increased shoot to root translocation of carbohydrates frequently observed in higher plants, is not related with a generally increased root exudation from the whole root system. Moreover, the CO_2-mediated stimulation of root exudation in apical root zones of both model plants depends on the nutritional status and developmental stage of the plant. Therefore, future approaches for quantifying or modelling plant-derived C inputs into soil under global climate change require a detailed understanding and consideration of rhizosphere processes to obtain reliable data.

The applied localized collection techniques for root exudates and rhizosphere soil at the same spatial and temporal resolution allowed exudation from specific root zones to be linked with microbial properties in the adhering soil. Apart from the specific interactions related with the *Rhizobium* symbiosis, elevated CO_2 had no effects on the abundance of total and denitrifying bacteria in the rhizosphere soil of apical root zones of bean. This contradicts our initial hypothesis that the rhizosphere microbial community profits from the additional release of plant-derived C from apical root zones. Moreover, despite the CO_2-induced alterations in PS exudation of barley, subsequent experiments in soil culture revealed no effects on the structural diversity of the rhizosphere bacterial community in apical root zones. The absence of detectable CO_2 effects may be attributed to the marginal and/or inconsistent stimulation of root exudation due to CO_2 elevation during plant growth. It remains to be established whether the restriction of PS release to local short-term pulses in the apical root zones of barley (to counteract microbial degradation; Neumann and Römheld, 2007) is responsible for the limited expression of effects on rhizosphere microorganisms. In conclusion, our results revealed that future trends of increasing atmospheric CO_2 concentration will probably not result in major shifts or growth of rhizosphere microbial communities. Nonetheless, the study with bean clearly reveals that rhizosphere microbial communities in apical root zones may adapt to the short-term additional supply of carbohydrates by altering enzyme regulation (production and activity). This could locally modify nutrient cycling in the adhering soil.

9.2 Effects of low-level herbivory by plant-parasitic nematodes

Beyond the impact of elevated atmospheric CO_2 concentrations, the final study of this thesis indicates that biotic factors such as belowground herbivory may also affect rhizosphere processes and hence plant growth. Low-level root infestation by *Meloidogyne incognita* promoted host plant growth in barley at an infection rate of approximately 41 root knots per plant. Depending on the developmental stage of the plant-nematode interaction, low-level root infestation by *M. incognita* increased rhizodepostion of the host plant. This occurred directly via leakage from damaged plant cells or indirectly via a modified root hair morphology of uninfected root zones, which affect root exudation. These short-term local and systemic alterations in rhizodepositon potentially impact microbial communities in rhizosphere soil. In an accompanied experiment, Poll et al. (2007) observed stimulatory effects on both bacteria and fungi at an early stage of the plant-nematode interactions. Thus, low-level herbivory by *M. incognita* may affect nutrient dynamics in the rhizosphere soil and hence plant nutrient uptake, as demonstrated by increased shoot P and N contents. This in turn may affect plant performance and competition. The possibility of a beneficial effect of low-level herbivory by plant-parasitic nematodes on host plant growth is particularly tempting, considering their traditional role as pests in agricultural soils, and is thus interesting for management strategies directed at manipulating soil biota. Recent results of Mao et al. (2007) suggest that bacterial-feeding nematodes also affect root morphology through hormonal effects. The impact of such modifications on C flow from roots to rhizosphere microbes is an interesting topic for future research in rhizosphere ecology.

10 References

Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO_2 enrichment? A meta-analytic review of the responses of photosysnthesis, canopy properties and plant production to rising CO_2. New Phytologist 165, 351-372.

Allard, V., Robin, C., Newton, P.C.D., Lieffering, M., Soussana, J.F., 2006. Short and long-term effects of elevated CO_2 on *Lolium perenne* rhizodepostion and its consequences on soil organic matter turnover and plant N yield. Soil Biology & Biochemistry 36, 1178-1187.

Amthor, J.S., 2001. Effects of atmospheric CO_2 concentration on wheat yield: review of results from experiments using various approaches to control CO_2 concentration. Field Crops Research 73, 1-34.

Baggs, E.M., Richter, M., Cadisch, G., Hartwig, U.A., 2003. Denitrification in grass swards is increased under elevated atmospheric CO_2. Soil Biology & Biochemistry 35, 729-732.

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

Bardgett, R.D., Denton, C.S., Cook, R., 1999b. Below-ground herbivory promotes soil nutrient transfer and root growth in grassland. Ecology Letters 2, 357-360.

Bardgett, R.D., Hobbs, P.J., Frostegård, Å., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management on an upland grassland. Biology and Fertility of Soils 22, 261-264.

Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84, 2258-2268.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground food webs: how plant responses to foliar herbivory influence soil organisms. Soil Biology & Biochemistry 30, 1867-1878.

Barker, A.V., 1999. Ammonium accumulation and ethylene evolution by tomato infected with root-knot nematode and grown under different regimes of plant nutrition. Communications in Soil Science and Plant Analysis 30, 175-182.

Barnard, R., Barthes, L., Le Roux, X., Leadley, P.W., 2004. Dynamics of nitrifying activities, denitrifying activities and nitrogen in grassland mesocosms as altered by elevated CO_2. New Phytologist, 162, 365-376.

Bazot, S., Ulff, L., Blum, H., Nguyen, C., Robin, C., 2006. Effects of elevated CO_2 concentration on rhizodeposition from *Lolium perenne* grown on soil exposed to 9 years of CO_2 enrichment. Soil Biology & Biochemistry 38, 729-736

Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., Scheu, S., 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biology & Biochemistry 29, 1023-1032.

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

Bird, D.M., 2004. Signaling between nematodes and plants. Current Opinion in Plant Biology 7, 372-376.

Bird, D.M., Koltai, H., 2000. Plant parasitic nematodes: habitats, hormones, and horizontally-acquired genes. Journal of Plant Growth Regulation 19, 183-194.

Blakeney, A.B., Mutton, L.L., 1980. A simple colorimetric method for the determination of sugars in fruits and vegetables. Journal of the Science of Food and Agriculture 31, 889-897.

Boeuf-Tremblay, V., Plantureux, S., Guckert, A., 1995. Influence of mechanical impedance on root exudation of maize seedlings at two developmental stages. Plant and Soil 172, 279-287.

Bolaños-Vásquez, M.C., Werner, D., 1997. Effects of *Rhizobium tropici*, *R. etli*, and *R. leguminosarum* bv. *phaseoli* on *nod* Gene – inducing flavonoids in root exudates of *Phaseolus vulgaris*. Molecular Plant-Microbe Interactions 10 (3), 339-346.

Bonkowski, M., 2004. Protozoan and plant growth: the microbial loop in soil revisited. New Phytologist 162, 617-631.

Brimecombe, M.J., De Leij, F.A.A.M., Lynch, J.M. 2007. Rhizodeposition and Microbial Populations. In: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 41-93.

Bruce, K.D., Jones, T.H., Bezemer, T.M., Thompson, L.J., Ritchie, D.A., 2000. The effect of elevated atmospheric carbon dioxide levels on soil bacterial communities. Global Change Biology 6, 427-434.

Burth, I., Ottow, J.C.G., 1983. Influence of pH on the production of N_2O and N_2 by different denitrifying bacteria and *Fusarium solani*. In: Hallberg, L. (Ed.), Environmental Biogeochemistry. Ecological Bulletins 35. FRN, Stockholm, pp. 207-215.

Byrd, D.W., Kirkpatrick, T., Barker, K.R., 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15, 142-143.

Cabrerizo, P.M., González, E.M., Aparicio-Tejo, P.M., Aresse-Igor, C., 2001. Continuous CO_2 enrichment leads to increased nodule biomass, carbon availability to nodules and activity of carbon-metabolising enzymes but does not enhance specific nitrogen fixation in pea. Physiologia Plantarum 113, 33-40.

Cardon, Z.G., 1996. Influence of rhizodeposition under elevated CO_2 on plant nutrition and soil organic matter. Plant and Soil 187, 277-288.

Carnol, M., Hogenboom, L., Jach, M.E., Remacle, J., Ceulemans, R., 2002. Elevated atmospheric CO_2 in open top chambers increases net nitrification and potential denitrification. Global Change Biology 8, 590-598.

Castells, E., Roumet, C., Penuelas, J., Roy, J., 2002. Intraspecific variability of phenolic concentrations and their responses to elevated CO_2 in two mediterranean perennial grasses. Environmental and Experimental Botany 47, 205-216.

Chèneby, D., Philippot, L., Hartmann, A., Hènault, C.F., Germon, J.C., 2000. 16S rRNA analysis for characterization of denitrifying bacteria isolated from three agricultural soils. FEMS Microbiology Ecology 34, 121-128.

Cheng, W., 1999. Rhizosphere feedbacks in elevated CO_2. Tree Physiology 19, 313-320.

Cheng, W., Johnson, D.W., 1998. Elevated CO_2, rhizosphere processes, and soil organic matter decomposition. Plant and Soil 202, 167-174.

Chishaki, N., Horiguchi T., 1997. Responses of secondary metabolism in plants to nutrient deficiency. Soil Science and Plant Nutrition 43,987-991.

Clays-Josserand, A., Lemanceau, P., Philippot, L., Lensi, R., 1997. Influence of two plant species (flax and tomato) on the distribution of nitrogen dissimilative abilities within fluorescent *Pseudomonas* spp. Applied and Environmental Microbiology 61, 1745-1749.

Cohn, E., Koltai, H., Sharon, E., Speigel, Y., 2002. Root-nematode interactions: recognition and pathogenicity. In: Waisel, J., Eshel, A., Kalkaf, U., (Eds.), Plant roots – the hidden half. Marcel Dekker, New York, USA, pp. 783-796.

Cramer, W., Bondeau, A., Woodward, F.I., Prentice, I.C., Betts, R.A., Brovkin, V., Cox, P.M., Fisher, V., Foley, J.A., Friend, A.D., Kucharik, C., Lomas, M.R., Ramankutty, N., Sitch, S., Smith, B., White, A., Young-Molling, C., 2001. Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. Global Change Biology 7, 357-373

Crowley, D., 2000. Function of siderophores in the rhizosphere. In: Pinton, R., Varanini, Z., Nannipieri, P., (Eds.), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 223-261.

Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S.L., Briat, J.F., Walker, E.L., 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. Nature 18, 409(6818), 346-349.

Dakora, F.D., Phillips, D.A., 1996. Diverse functions of isoflavanoids in legumes transcend anti-microbial definitions of phytoalexins. Physiological and Molecular Plant Pathology 49, 1-20.

Darwent, M.J., Paterson, E., McDonald, A.J.S., Tomos, A.D., 2003. Biosensor reporting of root exudation from *Hordeum vulgare* in relation to shoot nitrate concentration. Journal of Experimental Botany 54, 325-334.

Deiglmayr, K., Philippot, L., Hartwig, U.A., Kandeler, E., 2004. Structure and activity of the nitrate-reducing community in the rhizosphere of *Lolium perenne* and *Trifolium repens* under elevated atmospheric pCO_2. FEMS Microbiology Ecolology 49, 445-454.

Denton, C.S., Bardgett, R.D., Cook, R., Hobbs, P.J., 1999. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biology & Biochemistry 31, 155-165.

Dhillion, S.S., Roy, J., Abrams, M., 1996. Assessing the impact of elevated CO_2 on soil microbial activity in a Mediterranean model system. Plant and Soil 187, 333-342.

Diaz, S., Grime, J.P., Harris, J., McPherson, E., 1993. Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364, 616-617.

Dinkelaker, B., Hengeler, C., Neumann, G., Eltrop, L., Marschner, H., 1997. Root exudates and mobilization of nutrients. In: Rennenberg, H., Eschrich, W., Ziegler, H. (Eds.), Trees - Contributions to modern tree physiology. Backhuys Publishers, Leiden, Netherlands, pp. 441-452.

Drake, B.G., Gonzàlez-Meler, M.A., Long, S.P., 1997**.** More efficient plants: a consequence of rising atmospheric CO_2? Annual Review of Plant Physiology and Plant Molecular Biology 48, 609-639.

Drissner, D., Blum, H., Tscherko, D., Kandeler, E., 2007. Nine years of enriched CO_2 changes the function and structural diversity of soil microorganisms in a grassland. European Journal of Soil Science 58, 260-269.

Dromph, K.M., Cook, R., Ostle, N.J., Bardgett, R.D., 2006. Root parasite induced nitrogen transfer between plants is density dependent. Soil Biology & Biochemistry 38, 2495-2498.

Eamus, D., 2000. Some tree responses to CO_2 enrichment. In: Singh, S.N., (Ed.), Trace gas emissions and plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 75-95.

Ebersberger, D., Niklaus, P.A., Kandeler, E., 2003. Long term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. Soil Biology & Biochemistry 35, 965-972.

Ebersberger, D., Wermbter, N., Niklaus, P.A., Kandeler, E., 2004. Effects of long term CO_2 enrichment on microbial community structure in a calcareous grassland. Plant and Soil 264, 313-323.

Enwall, K., Philippot, L., Hallin, S., 2005. Activity and composition of the bacterial community respond differently to long-term fertilization. Applied and Environmental Microbiology 71, 8335-8343.

Erenoglu, B., Cakmak, I., Marschner, H., Römheld, V., Eker, S., Daghan, H., Kalayci, M., Ekiz, H., 1996. Phytosiderophore release does not relate well with Zn efficiency in different bread wheat genotypes. Journal of Plant Nutrition 19, 1569-1580.

Evans, R., 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78, 9-19.

Fangmeier, A., Grüters, U., Hertstein, U., Sandhage-Hofmann, A., Vermehren, B., Jäger, H.J., 1996. Effects of elevated CO_2, nitrogen supply and tropospheric ozone on spring wheat. I growth and yield. Environmental Pollution 91, 381-390.

Feng, Z., Dyckmans, J., Flessa, H., 2004. Effects of elevated carbon dioxide concentration on growth and N_2 fixation of young *Robinia pseudoacacia*. Tree Physiology 24, 323-330.

Ford, B.J., 1986. Even plants excrete. Nature 323, 763-763.

Freeman, C., Kim, S.Y., Lee, S.H., Kang, H., 2004. Effects of elevated atmospheric CO_2 concentration on soil microorganisms. The Journal of Microbiology 42, 267-277.

Fromin, N., Tarnawski, S., Roussel-Delif, L., Hamelin, J., Baggs, E.M., Aragno, M., 2005. Nitrogen fertiliser rate affects the frequency of nitrate-dissimilating *Pseudomonas* spp. in the rhizosphere of *Lolium perenne* grown under elevated pCO_2 (Swiss FACE). Soil Biology & Biochemistry 37, 1962-1965.

Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils, 22, 59-65.

Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry 25, 723-730.

Gericke, S., Kumis, B., 1952. Die kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. Zeitschrift für Pflanzenernährung und Bodenkunde 59, 235-247.

Ghiglione, J.F., Gourbière, F., Potier, P., Philippot, L., Lensi, R., 2000. Role of respiratory nitrate reductase in ability of *Pseudomonas fluorescence* YT101 to colonize the rhizosphere of maize. Applied and Environmental Microbiology 66, 4012-4016.

Gill, R.A., Polley, H.W., Johnson, H.B., Anderson, L.J., Maherali, H., Jackson, R.B., 2002. Non-linear grassland responses to past and future atmospheric CO_2. Nature 417, 279-282.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84, 489-500.

Glazer, I., Orion, D., Apelbaum, A., 1983. Interrelationships between ethylene production, gall formation, and root-knot nematode development in tomato plants infected with *Meloidogyne javanica*. Journal of Nematology 15, 539-544.

Goellner, M., Wang, X., Davis, E.L., 2001. Endo-β-1,4-glucanase expression in compatible plant-nematode interactions. The Plant Cell 13, 2241-2255.

Gommers, F.J., Dropkin, V.H., 1977. Quantitative histochemistry of nematode induced transfer cells. Phytopathology 67, 869-873.

Göttlein, A., Hell, U., Blasek, R., 1996. A system for microscale tensiometry and lysimetry. Geoderma, 69, 147-156.

Graham, R.D., Webb, M.J., 1991. Micronutrients and disease resistance and tolerance in plants. In: Micronutrients in Agriculture, 2nd ed. Soil Science Society of America, SSSA Book Series No.4.

Grayston, S.J., Campbell, C.D., Lutze, J.L., Gifford, R.M., 1998. Impact of elevated CO_2 on the metabolic diversity of microbial communities in N-limited grass swards. Plant and Soil 203, 289-300.

Grayston, S.J., Dawson, L.A., Treonis, A.M., Murray, P.J., Ross, J., Reid, E.J., MacDougall, R., 2001. Impact of root herbivory by insect larvae on soil microbial communities. European Journal of Soil Biology 37, 277-280.

Griffiths, B.S., Ritz, K., Ebblewhite, N., Paterson, E., Killham, K., 1998. Ryegrass rhizosphere microbial community structure under elevated carbon dioxide concentrations, with observations on wheat rhizosphere. Soil Biology & Biochemistry 30, 315-321.

Haase, S., Neumann, G., Kania, A., Kuzyakov, Y., Römheld, V., Kandeler, E., 2007. Elevation of atmospheric CO_2 and N-nutritional status modify nodulation, nodule carbon supply, and root exudation of *Phaseolus vulgaris* L. Soil Biology & Biochemistry 39, 2208-2221.

Hamilton, III, E.W., Frank, D.A., 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82, 2397-2402.

Hammes, U.Z., Nielsen, E., Honaas, L.A., Taylor, C.G., Schachtman, D.P., 2006. AtCAT6, a sink-tissue-localized transporter for essential amino acids in *Arabidopsis*. The Plant Journal 48, 414-426.

Hammes, U.Z., Schachtman, D.P., Berg, R.H., Nielsen, E., Koch, W., McIntyre, L.M., Taylor C.G., 2005. Nematode-induced changes of transporter gene expression in *Arabidopsis* roots. Molecular Plant-Microbe Interactions 18, 1247-1257.

Hartwig, U.A., Lüscher, A., Daepp, M., Blum H., Soussana J.-F., Nösberger, J., 2000. Due to symbiotic N_2 fixation, five years of elevated atmospheric pCO_2 had no effect on the N concentration of plant litter in fertile, mixed grassland. Plant and Soil 224, 43-50.

Henry, F., Nguyen, C., Paterson, E., Sim, A., Robin, C., 2005. How does nitrogen availability alter rhizodeposition in *Lolium multiflorum* Lam. during vegetative growth? Plant and Soil 269,181-191.

Henry, H.A., Juarez, J.D., Field, C.B., Vitousek, P.M., 2005. Interactive effects of elevated CO_2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. Global Change Biology 11, 1808-1815.

Henry, S., Baudoin, J.C., López-Gutiérrez, Martin-Laurent, F., Brauman, A., Philippot L., 2004. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. Journal of Microbiological Methods 59, 327-335. (Erratum, 61, 289-290)

Henry, S., Bru, D., Stres, B., Hallet, S., Philippot, L., 2006. Quantitative detection of the *nosZ* gene, encoding the nitrous oxide reductase, and comparison of the abundance of 16S rRNA, *narG*, *nirK* and *nosZ* genes in soils. Applied and Environmental Microbiology 72, 5181-5189.

Hodge, A., Millard, P., 1998. Effect of elevated CO_2 on carbon partitioning and exudate release from *Plantago lanceolata* seedlings. Physiologia Plantarum 103, 280-286.

Hodge, A., Paterson, E., Grayston, S.J., Campbell, C.D., Ord, B.G., Killham, K., 1998. Characterization and microbial utilisation of exudate material from the rhizosphere of *Lolium perenne* grown under CO_2 enrichment. Soil Biology & Biochemistry 30, 1033-1043.

Højberg, O., Binnerup, S.J., Sørensen, J., 1996. Potential rates of ammonium oxidation, nitrite oxidation, nitrate reduction and denitrification in the young barley rhizosphere. Soil Biology & Biochemistry 28, 47-54.

Holland, J.N., Cheng, W., Crossley, D.A. Jr., 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. Oecologia 107, 87-94.

Hoosbeek, M.R., Lukac, M., Godbold, D.L., Velthorst, E.J., Biondi, F.A., Peressotti, A., Cotrufo, M.F., De Angelis, P., Scarascia-Mugnozza, G., 2004., More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): cause of increased priming effect? Global Biochemical Cycles, 18, GB1040 (1-7).

Hördt, W., Römheld, V., Winkelmann, G., 2000. Fusarinines and dimerum acid, mono- and dihydroxamate siderophores from *Penicillium chrysogenum*, improve iron utilization by strategy I and strategy II plants. BioMetals 13, 37-46.

Hu, S., Wu, J., Burkey, K.O., Firestone, M.K., 2005. Plant and microbial N acquisition under elevated atmospheric CO_2 in two mesocosm experiments with annual grasses. Global Change Biology 11, 213-223.

Hungate, B.A., Holland, E.A., Jackson, R.B., Chapin III, F.S., Mooney, H.A., Field, C.B., 1997. The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388, 576-579.

Hungate, B.A., Jaeger, C.H., Gamara, G., Chapin, F.S.III, Field C.B., 2000. Soil microbiota in two annual grasslands: responses to elevated atmospheric CO_2. Oecologia 124, 589-598.

Husted, S., Hebbern, C.A., Mattsson, M., Schjoerring, J.K., 2000. A critical experimental evaluation of methods for determination of NH_4^+ in plant tissue, xylem sap and apoplastic fluid. Physiologia Plantarum 109, 167-179.

IPCC, Intergovernmental Panel on Climate Change, (Eds.), 2001. Climate Change 2001: Third Assessment Report. Cambridge University Press, Cambridge, UK.

IPCC, Intergovernmental Panel and Climate Change, (Eds.), 2007. Climate Change 2007: Fourth Assessment Report. Cambridge University Press, Cambridge, UK.

Jaubert, S., Laffaire, J.B., Abad, P., Rosso, M.N., 2002. A polygalacturonase of animal origin isolated from the root-knot nematode *Meloidogyne incognita*. FEBS Letters 522, 109-112.

Joergensen, R.G., Müller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EN} value. Soil Biology & Biochemistry 28, 33-37.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. Soil Biology & Biochemistry 28, 25-31.

Jones, D.L., 1998. Organic acids in the rhizosphere – A critical review. Plant and Soil 2005, 25-44.

Jones, D.L., 1999. Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biology & Biochemistry 31, 613-622.

Jones, D.L., Hodge, A., Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163, 459-480.

Jones, D.L., Owen, A.G., Farrar, J.F., 2002 Simple method to enable the high resolution determination of total free amino acids in soil solution and soil extracts. Soil Biology & Biochemistry 34, 1893-1902.

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., Philippot, L., 2006 a. Abundance of *narG*, *nirS*, *nirK*, and *nosZ* genes of denitrifying bacteria during primary successions of a glacier foreland. Applied and Environmental Microbiology 72, 5957-5962.

Kandeler, E., Marschner, P., Tscherko, D., Gahoonia, T.S., Nielsen, N.E., 2002. Microbial community composition and functional diversity in the rhizosphere of maize. Plant and Soil 238, 301-312.

Kandeler, E., Mosier, A.R., Morgan, J.A., Milchunas, D.G., King, J.Y., Rudolph, S., Tscherko, D., 2006 b. Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. Soil Biology & Biochemistry 38, 2448-2460.

Kang, H.J., Freemann, C., Ashendon, T.W., 2001. Effects of elevated CO_2 on fen peat biogeochemistry. Science of the Total Environment 279, 45-50.

Kang, H.J., Kim, S.Y., Fenner, N., Freeman, C., 2005. Shifts of soil enzyme activities in wetlands exposed to elevated CO_2. Science of the Total Environment 337, 207-212.

Kape, R., Wex, K., Parniske, M., Görge, E., Wetzel, A., Werner, D., 1992. Legume root metabolites and VA-mycorrhiza development. Journal of Plant Physiology 141, 54-60.

Keeling, C.D., Whorf, T.P., 2003. Atmospheric CO_2 concentrations (ppmv) derived from *in situ* air sampoles collected at Mauna Loa Observatory, Hawaii, Scripps Institution of Oceanography (SIO), University of California, La Jolla, California USA 92093-0444. http://cdiac.esd.ornl.gov/ftp/ndp001/maunaloa.co2.

Kennedy, M.J., Niblack, T.L., Krishnan, H.B., 1999. Infection by *Heterodera glycines* elevates isoflavonoid production and influences soybean nodulation. Journal of Nematology 31, 341-347.

Kettunen, R., Saarnio, S., Martikainen, P., Silvola, J., 2005. Elevated CO_2 concentrations and nitrogen fertilisation effects on N_2O and CH_4 fluxes and biomass production of *Phleum pratense* on farmed peat soil. Soil Biology & Biochemistry 37, 739-750.

Khan, W., 1993. Nematode Interactions. Chapman and Hall, London, UK.

Kim, Y.S., 2002. Malonate metabolism: biochemistry, molecular biology, physiology, and industrial application. Journal of Biochemistry and Molecular Biology 35, 443-451.

Kim, Y.S., Kang, S.W., 1994. Novel malonamidases in *Bradyrhizobium japonicum*. Journal of Biological Chemistry 269, 8014-8021.

Kimball, B.A., Kobayashi, K., Bindi, M., 2002. Responses of agricultural crops to free-air CO_2 enrichment. Advances in Agronomy 77, 293-368.

Klamer, M., Roberts, M.S., Levine, L.H., Drake, B.G., Garland, J.L., 2002. Influence of elevated CO_2 on the fungal community in a coastal scrub oak forest soil investigated with terminal-restriction fragment length polymorphism analysis. Applied and Environmental Microbiology 68, 4370-4376.

Klemedtsson, L., Berg, P., Clarholm, M., Schnürer, J., Roswall, T., 1987. Microbial nitrogen transformations in the root environment of barley. Soil Biology & Biochemistry 19, 551-558.

Körner, C., 1996. The response of complex multispecies systems to elevated CO_2. In: Walker, B., Steffen, W., (Eds.), Global Change and Terrestrial Ecosystems. Cambridge University Press, Cambridge, pp. 21-42.

Körner, C., 2000. Biosphere responses to CO_2 enrichment. Ecological Applications 10, 1590-1619.

Kuzyakov, Y, Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-431.

Kuzyakov, Y., 2002. Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. Soil Biology & Biochemistry 24, 1621-1631.

Kuzyakov, Y., 2006. Root exudates and organic composition of plant roots. In: Luster, J., Finlay, R., (Eds.), Handbook of Methods used in Rhizosphere Research. Swiss Federal Research Institute WSL, Birmensdorf, Switzerland, pp. 309-312.

Kuzyakov, Y., Raskatov, A., Kaupenjohann, M., 2003. Turnover and distribution of root exudates of *Zea mays*. Plant and Soil 254, 317-327.

Kuzyakov, Y., Shevtzova, E., Pustovoytov, K., 2006. Carbonate re-crystallization in soil revealed by ^{14}C labeling: Experiment, model and significance for paleo-environmental reconstructions. Geoderma 131, 45-58.

Larson, J.L., Zak, D.R., Sinsabaugh, R.L., 2002. Extracellular enzyme activity beneath temperature trees growing under elevated carbon dioxide and ozone. Soil Science Society of America Journal 66, 1848-1856.

Li, J., Copeland, L., 2000. Role of malonate in chickpeas. Phytochemistry 54, 585-589.

Liljeroth, E., Kuikman, P., Van Veen, J.A., 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant and Soil 161, 233-240.

Linne von Berg, K.H., Bothe, H., 1992. The distribution of denitrifying bacteria in soil monitored by DNA-probing. FEMS Microbiology Ecology 86, 331-340.

Lüscher, A., Hartwig U.A., Suter, D., Nosberger, J., 2000. Direct evidence that symbiotic N_2 fixation in fertile grassland is an important trait for a strong response of plants to elevated atmospheric CO_2. Global Change Biology 6, 655-662.

Lussenhop, J., Fogel, R., 1991. Soil invertebrates are concentrated on roots. In: Keiser, D.L., Cregan, P.B., (Eds.), The Rhizosphere and Plant Growth. Kluwer Academic Publishers, Boston, MA, p. 111.

Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant and Soil 129, 1-10.

Ma, J.F., Nomoto, K., 1996. Effective regulation of iron acquisition in graminaceous plants. The role of mugineic acids as phytosiderophores. Physiologia Plantarum 97, 609-617.

Manzanilla-López, R.H., Evans, K., Bridge, J., 2003. Plant diseases caused by nematodes. In: Chen, Z.X., Chen, S.Y., Dickson, D.W., (Eds.), Nematology. Advances and perspectives. Volume 2. Nematode Management and Utilization. CABI Publishing, Wallingford, UK, pp. 637-703.

Mao, X., Hu, F., Griffiths, B., Chen, X., Liu, M., Li, H., 2007. Do bacterial-feeding nematodes stimulate root proliferation through hormonal effects? Soil Biology & Biochemistry 39, 1816-1819.

Marilley, L., Hartwig, U.A., Aragno, M., 1999. Influence of an elevated atmospheric CO_2 content on soil and rhizosphere bacterial communities beneath *Lolium perenne* and *Trifolium repens* under field conditions. Microbial Ecology 38, 39-49.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London, UK.

Marschner, H., Römheld, V., 1994. Strategies of plants for acquisition of iron. Plant and Soil 165, 261-274.

Marschner, H., Römheld, V., Kissel, M., 1986 Different strategies in higher plants in mobilization and uptake of iron. Journal of Plant Nutrition 9, 695-713.

Marschner, H., Römheld, V., Kissel, M., 1987. Localization of phytosiderophore release and iron uptake along intact barley roots. Physiologia Plantarum 71, 157-162.

Marschner, P., Crowley, D.E., 1998. Phytosiderophores decrease iron stress and pyroverdine production of *Pseudomonas fluorescens* PF-5 (PVD-INAZ). Soil Biology & Biochemistry 9, 1275-1280.

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.

Martens D.C., Lindsay, W.L., 1990. Testing soils for copper, iron, manganese and zinc. In: Westerman, R.L., (Ed.), Soil testing and plant analysis 3rd ed. Soil science Society of America, Madison, Wisconsin, USA., pp. 229-264

Martín-Olmedo, P., Rees, R.M., Grace, J., 2002. The influence of plants grown under elevated CO_2 and N fertilisation on soil nitrogen dynamics. Global Change Biology 8, 643-657.

Marx, M.C., M. Wood, Jarvis, S.C., 2001. A microplate fluorometric assay for the study of enzyme diversity in soils. Soil Biology & Biochemistry 34, 1647-1654.

Mawsdley, J.L., Bardgett, R.D., 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the microbial population of an upland grassland soil. Biology and Fertility of Soils 24, 52-58.

Mayr, C., Miller, M., Insam, H., 1999. Elevated CO_2 alters community-level physiological profiles and enzyme activities in alpine grassland. Journal of Microbiological Methods 36, 35-43.

McClure, M.A., 1977. *Meloidogyne incognita*: A metabolic sink. Journal of Nematology 9, 88-90.

Meharg, A.A., 1994. A critical review of labelling techniques used to quantify rhizosphere carbon flow. Plant and Soil 166, 55-62.

Michael, G., 2001. The control of root hair formation: suggested mechanisms. Journal of Plant Nutrition and Soil Science. 164, 111-119.

Monje, O.A., Bugbee, B., 1992. Inherent limitations of non destructive chlorophyll meters: A comparison of two types of meters. Horticultural Science 27, 69-71.

Montealegre, C.M., van Kessel, C., Blumenthal, J.M., Hur, H.G., Hartwig, U.A., Sadowsky, M.J., 2000. Elevated atmospheric CO_2 alters microbial population structure in a pasture ecosystem. Global Change Biology 6, 475-482.

Montealegre, C.M., van Kessel, C., Ruselle, M.P., Sadowsky, M.J., 2002. Changes in microbial activity and composition in a pasture ecosystem exposed to elevated atmospheric carbon dioxide. Plant and Soil 243, 197-207.

Mori, S., Nishizawa, N., Kawai, S., Sato, Y., Takagi, S., 1987. Dynamic state of mugineic acid and analogous phytosiderophores in Fe-deficient barley. Journal of Plant Nutrition 10, 1003-1011.

Mulvaney, R.L., Khan, S.A., Mulvaney, C.S., 1997. Nitrogen fertilizers promote denitrification. Biology and Fertility of Soils 24, 211-220.

Neumann, G., 2006. Root exudates and organic composition of plant roots. In: Luster, J., Finlay, R., (Eds.) Handbook of Methods used in Rhizosphere Research. Swiss Federal Research Institute WSL, Birmensdorf, Switzerland, pp. 317-320.

Neumann, G., 2007. Root exudates and organic composition of plant roots. In: Luster, J., Finlay, R. (ED.) Handbook of Methods used in Rhizosphere Research. Swiss Federal Institute for Forest, Snow, and Landscape Research, Birmensdorf, Switzerland, published online, http://www.rhizo.at.

Neumann, G., Haake, C., Römheld, V., 1999 a. Improved HPLC method for determination of phytosiderophores in root washings and tissue extracts. Journal of Plant Nutrition 22, 1389-1402.

Neumann, G., Massonneau, A., Martinoia, E., Römheld, V., 1999 b. 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., Römheld, V., 2000. The release of root exudates as affected by the plant physiological status. In: Pinton, R., Varanini, Z., Nannipieri, P., (Eds.), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 41-93.

Neumann, G., Römheld, V., 2002. Root induced changes in the availability of nutrients in the rhizosphere. In: Waisel, Y., Eshel, A., Kafkafi, U., (Eds.), Plant Roots: The hidden half. Marcel Dekker, New York, pp. 617-649.

Neumann, G., Römheld, V., 2007. The release of root exudates as affected by the plant physiological status. In: Pinton, R., Varanini, Z., Nannipieri, P., (Eds.), The Rhizosphere Biochemistry and Organic Substances at the Soil-Plant Interface. (Second Edition), Marcel Dekker, New York, pp. 23-72.

Nguyen, C., 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23, 375-396.

Niklaus, P.A., Spinnler, D., Körner, C., 1998. Soil moisture dynamics of calcareous grassland under elevated CO_2. Oecologia 117, 201-208.

Niklaus, P.A., Stocker, R., Körner, C., Leadley, P.W., 2000. CO_2 flux estimates tend to overestimate ecosystem C sequestration at elevated CO_2. Functional Ecology 14, 546-559.

Nishizawa, N.K., Mori, S., 1987. The particular vesicles appearing in barley root cells and its relation to mugineic acid secretion. Journal of Plant Nutrition 10, 1013-1020.

Norby, R.J. 1994. Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. Plant and Soil 165, 9-20.

Nowak, R.S., Ellsworth, D.S., Smith, S.D., 2004. Functional responses of plants to elevated atmospheric CO_2 – do photosynthetic and productivity data from FACE experiments support early predictions? New Phytologist 162, 253-280.

Orion, D., Kritzman, G., Meyer, S.L.F., Erbe, E.F., Chitwood, D.J., 2001. A role of the gelatinous matrix in the resistance of root-knot nematode (*Meloidogyne* spp.) eggs to microorganisms. Journal of Nematology 33, 203-207.

Paterson, E., Hall, J.M., Rattray, E.A.S., Griffiths, B.S., Ritz, K., Killham, K., 1997. Effect of elevated CO_2 on rhizosphere carbon flow and soil microbial processes. Global Change Biology 3, 363-377.

Peltonen, P.A., Vapaavuori, E., Julkunen-Tiitto, R., 2005. Accumulation of phenolic compounds in birch leaves is changed by elevated carbon dioxide and ozone. Global Change Biology 11, 1305-1324.

Pendall, E., Bridgham, S., Hanson, P.J., Hungate, B., Kicklighter, D.W., Johnson, D.W., Law, B.E., Luo, Y., Megonigal, J.P., Olsrud, M., Ryan, M.G., Wan, S., 2004a. Belowground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytologist 162, 311-322.

Pendall, E., Mosier, A.R., Morgan, J. A., 2004. Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. New Phytologist 162, 447-458.

Philippot, L., Hallin, S., Schloter, M., 2007. Ecology of denitrifying prokaryotes in agricultural soil. Advances in Agronomy, in press.

Piha, M.I., Munns, D.N., 1987. Nitrogen fixation potential of beans (*Phaseolus vulgaris* L.) compared with other grain legumes under controlled conditions. Plant and Soil 98, 169-182.

Pitts, R.J., Cernac, A., Estelle, M., 1998. Auxin and ethylene promote root hair elongation in *Arabidopsis*. The Plant Journal 16, 553-560.

Plaxton, WC. 1998. Metabolic aspects of phosphate starvation in plants. In: Lynch, J.P., Deikman, J., (ED.), Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic, and ecosystem processes. American Society of Plant Physiologists. pp. 229-241.

Poll, C., Ingwersen, J., Stemmer, M., Gerzabek, M.H., Kandeler, E., 2006. Mechanisms of solute transport affect small-scale abundance and function of soil microorganisms in the detritusphere. European Journal of Soil Science 57, 583-595.

Poll, J., Marhan, S., Haase, 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.

Reuter, D.J., Robinson, J.B., 1997. Plant analysis. An interpretation manual. 2nd ed., CSIRO Publishing, Collingwood, Australia.

Richter, M., Hartwig, U.A., Frossard, E., Nösberger, J., Cadisch, G., 2003. Gross fluxes of nitrogen in grassland soil exposed to elevated atmospheric pCO_2 for seven years. Soil Biology & Biochemistry 35, 1325-1335.

Ridge, R.W., Katsumi, M., 2002. Root hairs: Hormones and tip molecules. In: Waisel, Y., Eshel, A., Kafkafi, U., (Eds.), Plant roots – the hidden half. Marcel Dekker, New York, USA, pp. 83-91.

Rogers, H.H., Runion, G.B., Krupa, S.V., 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. Environmental Pollution. 83, 155-189.

Römheld, V. 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: An ecological approach. Plant and Soil 130, 127-134.

Roussel-Delif, L., Tarnawski, S., Hamelin, J., Philippot, L., Aragno, M., Fromin, N., 2005. Frequency and diversity of nitrate reductase genes among nitrate-dissimilating *Pseudomonas* in the rhizosphere of perennial grasses grown in field conditions. Microbial Ecology 49, 63-72.

Ruess, L., 1995. Studies on the nematode fauna of an acid forest soil: spatial distribution and extraction. Nematologica 41, 229-239.

Sadowsky, M.J., Schortemeyer, M., 1997. Soil microbial responses to increased concentrations of atmospheric CO_2. Global Change Biology 3, 217-224.

Sakaguchi, T., Nishizawa, N.K., Nakanishi, H., Yoshimura, E., Mori, S., 1999. The role of potassium in the secretion of mugineic acids family phytosiderophores from iron-deficient barley roots. Plant and Soil 215, 221-227.

Salsman, K.J., Jordan, D.N., Smith, S.D., Neuman D.S., 1999. Effect of atmospheric CO_2 enrichment on root growth and carbohydrate allocation of *Phaseolus* spp. International Journal of Plant Sciences 160(6), 1075-1081.

Sandnes, A., Eldhuset, T.D., Wollebæk, G., 2005. Organic acids in root exudates and soil solution of Norway spruce and silver birch. Soil Biolology & Biochemistry 37, 259-269.

Sasaki, T., Yamamoto, Y., Ezaki, B., Katsuhara, M., Ahn, S.J., Ryan, P.R., Delhaize, E., Matsumoto, H., 2004. A wheat gene encoding an aluminium-activated malate transporter, The Plant Journal 37, 645-653.

Sasser, J.N., 1980. Root-knot nematodes: A global menace to crop production. Plant Disease 64, 36-41.

Satterthwaite, F.E., 1946. An approximate distribution of estimates of variance components. Biometrics Bulletin 2, 110-114.

Sauerbeck, D., Johnen, B., 1976. Der Umsatz von Pflanzenwurzeln im Laufe der Vegetationsperiode und dessen Beitrag zur “Bodenatmung”. Zeitschrift für Pflanzenernährung und Bodenkunde 139, 315-328.

Scheu, S., 1992. Automated measurement of the respiratory response of soil microcompartments: active microbial biomass in earthworm faeces. Soil Biology & Biochemistry, 24, 1113-1118.

Schilling, G., Gransee, A., Deubel, A., Lesovic, G., Ruppel, S., 1998. Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 465-478.

Schortemeyer, M., Atkin, O.K., McFarlane, N., Evans, J.R., 2002. N_2 fixation by *Acacia* species increases under elevated atmospheric CO_2. Plant, Cell and Environment 25, 567-579.

Schortemeyer, M., Hartwig, U.A., Hendrey, G.R., Sadowsky, M., 1996. Microbial community changes in the rhizosphere of white clover and perennial ryegrass exposed to free-air carbon dioxide enrichment (FACE). Soil Biology & Biochemistry 28, 1717-1724.

Schüller, H. 1969. Die CAL-Methode zur Bestimmung des pflanzenverfügbaren Phosphates in Böden. Zeitschrift für Pflanzenernährung und Bodenkunde 123, 48-63. (in German)

Schultze, M., Kondorosi, A., 1998. Regulation of symbiotic root nodule development. Annual Reviews of Genetics 32, 33-57.

Sharma, S., Aneja; M.K., Mayer, J., Munch, J.C., Schloter, M., 2005. Diversity of transcripts of nitrate reductase genes (*nirK* and *nirS*) in rhizospheres of grain legumes. Applied and Environmental Microbiology, 71, 2001-2007.

Shaw, L.J., Morris, P., Hooker, J.E., 2006, Perception and modification of plant flavonoid signals by rhizosphere microorganisms. Environmental Microbiology 8, 1867-1880.

Šimek, M., Cooper, J.E., 2002. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. European Journal of Soil Science 53, 345-354.

Smart, D.R., Ritchie, K., Stark, J.M., Bugbee, B., 1997. Evidence that elevated CO_2 levels can indirectly increase rhizosphere denitrifier activity. Applied and Environmental Microbiology 63, 4621-4624.

Sonneman, I., Wolters, V., 2005. The micro food web of grassland soils responds to a moderate increase in atmospheric CO_2. Global Change Biology 11, 1148-1155.

Stephan, U.W., Rudolph, A., 1984. An improved and standardized method for extraction of nicotianamine from plant tissue. Biochemie und Physiologie der Pflanzen 179, 517-523.

Strasser O., Köhl, K., Römheld, V., 1999. Overestimation of apoplastic Fe in roots of soil grown plants. Plant and Soil 210, 179-187.

Suter, D., Frehner, M., Fischer, B.U., Nösberger, J., Lüscher, A., 2002. Elevated CO_2 increases carbon allocation to the roots of *Lolium perenne* under free-air CO_2 enrichment but not in a controlled environment. New Phytologist 154, 65-75.

Swain, T., Hillis, W.E., 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture. 10, 63-68.

Takagi, S, Kamei, S., Yu, M.H., 1988. Efficiency of iron extraction from soil by mugineic acid family phytosiderophores. Journal of Plant Nutrition 11, 643-651.

Takagi, S., 1976. Naturally occurring iron chelating compounds in oat- and rice-root washings. Journal of Plant Nutrition and Soil Science 22, 423-433.

Tang, C., McLay C.D.A., Barton, L., 1997. A comparison of proton excretion of twelve pasture legumes grown in nutrient solution. Australian Journal of Experimental Agriculture 37, 563-570.

Tegos, G., Stermitz, F.R., Lomovskaya, O., Lewis, K., 2002. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrobial Agents and Chemotherapy 46, 3133-3141.

Throbäck, I.N., Enwall, K., Jarvis, A., Hallin, S., 2004. Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiology Ecology 49, 401-417.

Tiedje, J.M., 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder, A.J. (Ed.), Biology of anaerobic microorganisms. John Wiley & Sons, Inc., New York, N.Y. p. 179–244.

Todorovic, C., Nguyen, C., Robin, C., Guckert, A., 2001. Root and microbial involvement in the kinetics of ^{14}C-partitioning to rhizosphere respiration after a pulse labelling of maize assimilates. Plant and Soil 228, 179-189.

Tu, C., Koenning, S.R., Hu, S., 2003. Root-parasitic nematodes enhance soil microbial activities and nitrogen mineralization. Microbial Ecology 46, 134-144.

Uselman, S.M., Qualls, R.G., Thomas, R.B., 2000. Effects of increased atmospheric CO_2, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). Plant and Soil 222, 191-202.

Van der Putten, W.H., Vet, L.E.M., Harvey, J.A., Wäckers, F.L., 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends in Ecology and Evolution 16, 547-554.

Van Ginkel, J.H., Gorissen, A., Polci, D., 2000. Elevated atmospheric carbon dioxide concentration: effects of increased carbon input in a *Lolium perenne* soil on microorganisms and decomposition. Soil Biology & Biochemistry 32, 449-456.

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.

Van Kessel, C., Horwath, W.R., Hartwig, U.A., Harris, D., Lüscher, A., 2000. Net soil carbon input under ambient and elevated CO_2 concentrations: isotopic evidence after four years. Global Change Biology 6, 435-444.

Van Veen, J.A., Liljeroth, E., Lekkerkerk, L.J.A., van de Geijn, S.C., 1991. Carbon fluxes in plant-soil systems at elevated atmospheric CO_2 levels. Ecological Applications 1, 175-181.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703-707.

Walker, T.S., 2003 Root exudation and rhizosphere biology. Plant Physiology, 132, 44-51.

Walter, A., Pich, A., Scholz, G., Marschner, H., Römheld, V., 1995a. Effects of iron nutritional status and time of day on concentrations of phytosiderophores and nicotianamine in different root and shoot zones of barley. Journal of Plant Nutrition 18, 1577-1593.

Walter, A., Pich, A., Scholz, G., Marschner, H., Römheld, V., 1995b. Diurnal variations in release of phytosiderophores and in concentrations of phytosiderophores and nicotianamine in roots and shoots of barley. Journal of Plant Physiology 147, 191-196.

Wang, E.L.H., Bergeson, G.B., 1974. Biochemical changes in root exudate and xylem sap of tomato plants infected with *Meloidogyne incognita*. Journal of Nematology 6, 194-202.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629-1633.

Wasaki, J., Rothe, A., Kania, A., Neumann, G., Römheld, V., Shinano, T., Osaki, M., Kandeler, E., 2005. Root exudation, P acquisition and microbial diversity in the rhizosphere of *Lupinus albus* as affected by P supply and atmospheric CO_2 concentration. Journal of Environmental Quality 34, 2157-2166.

Watanabe, S., Wada, H., 1989. Mugineic acid-decomposing bacteria isolated of iron-deficient barley. Jpn. Journal of Plant Nutrition and Soil Science 60, 413-417.

Webber, A.N., Nie, G.Y., Long, S.P., 1994. Acclimation of photosynthetic proteins to rising atmospheric CO_2 Photosynthesis Research 39, 413-425.

Werner, D., 2000. Organic signals between plants and microorganisms. In: Pinton, R., Varanini, Z., Nannipieri, P. (ED.), The rhizosphere: biochemistry and organic substances at the soil-plant interface. Marcel Dekker, New York, pp 197-222.

Werner, D., 2007. Molecular biology and ecology of the *Rhizobia*-legume symbiosis. In: Pinton, R., Varanini, Z., Nannipieri, P., (Eds.), The Rhizosphere Biochemistry and Organic Substances at the Soil-Plant Interface. (Second Edition), Marcel Dekker, New York, pp. 237-266.

Wiemken, V., Laczko, E., Ineichen, K., Boller, T., 2001. Effects of elevated carbon dioxide and nitrogen fertilization on mycorrhizal fine roots and the soil microbial community in Beech Spruce ecosystems on siliceous and calcareous soil. Microbial Ecology 42, 126-135.

Williams, M.A., Rice, C.W., Owensby, C.E., 2000. Carbon dynamics and microbial activity in tallgrass prairie exposed to elevated CO_2 for 8 years. Plant and Soil 227, 127-131.

Williamson, V.M., Gleason, C.A., 2003. Plant-Nematode interactions. Current Opinion in Plant Biology 6, 327-341.

Wirén, N. von, Römheld, V., Morel, J.L., Guckert, A., Marschner, H., 1993. Influence of microorganisms on iron acquisition in maize. Soil Biology & Biochemistry 25, 371-376.

Wirén, N. von, Römheld, V., Morel, J.L., Shiori, T., Marschner, H., 1995. Competition between microorganisms and roots of barley and sorghum for iron accumulated in the root apoplasm. New Phytologist 130, 511-521.

Wubben II, M.J.E, Su, H., Rodermel, S.R., Baum, T.J., 2001. Susceptibility to the sugar beet cyst nematodes is modulated by ethylene signal transduction in *Arabidopsis thaliana*. Molecular Plant-Microbe Interactions 14, 1206-1212.

Wubben II, M.J.E., Rodermel, S.R., Baum, T.J., 2004. Mutation of a UDP-glucose-4-epimerase alters nematode susceptibility and ethylene responses in *Arabidopsis* roots. The Plant Journal 40, 712-724.

Wurst, S., Langel, R., Rodger, S., Scheu, S., 2006. Effects of belowground biota on primary and secondary metabolites in *Brassica oleracea*. Chemoecology 16, 69-73.

Yamakawa, T., Kato, S., Ishida, K., Kodama, T., Minoda, Y. 1983. Production of anthocyanins by *Vitis* cells in suspension culture. Agricultural and Biological Chemistry 47, 2185-2191.

Yeates, G.W., Bardgett, R.D., Cook, R., Hobbs, P.J., Bowling, P.J., Potter, J.F., 1997. Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. Journal of Applied Ecology 34, 453-470.

Yeates, G.W., Newton, P.C.D., Ross, D.J., 2003. Significant changes in soil microfauna in grazed pasture under elevated carbon dioxide. Biology and Fertility of Soils 38, 319-326.

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.

Yehuda, Z., Shenker, M., Römheld, V., Marschner, H., Hadar, Y., Chen, Y., 1996. The role of ligand exchange in uptake of iron from microbial siderophores by graminaceous plants. Plant Physiology 112, 1273-1280.

Yurgel, S.N., Kahn, M.L. 2004. Dicarboxylate transport by rhizobia. FEMS Microbiological Reviews 28, 489-501.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., Teeri, J.A., Fogel, R., Randlett, D.L., 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant and Soil 151, 105-117.

Zak, D.R., Pregitzer, K.S., King, J.S., Holmes, W.E., 2000. Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytologist 147, 201-222.

Zanetti, S., Hartwig, U.A., Lüscher, A., Hebeisen, T., Frehner, M., Fischer, B.U., Hendrey, G.R., Blum, H., Nösberger, J., 1996. Stimulation of symbiotic N_2 fixation in *Trifolium repens* L. under elevated atmospheric pCO_2 in a grassland ecosystem. Plant Physiology 112, 575-583.

Zanetti, S., Hartwig, U.A., Nösberger, J., 1998. Elevated atmospheric CO_2 does not affect *per se* the preference for symbiotic nitrogen as opposed to mineral nitrogen of *Trifolium repens* L. Plant, Cell and Environment 21, 623-630.

Zelles, L., 1999. Fatty acid pattern of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111-129.

Zhang, W.H., Ryan, P.R., Tyerman, S.D., 2001. Malate-permeable channels and cation channels activated by aluminium in the apical cells of wheat roots. Plant Physiology 125, 1459-1472.

Zumft, W.G., 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61, 533-616.

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Haase, S., Kandeler, E., Kuzyakov, Y., Kania, A., Edelkott, I., Marschner, P., Römheld, V., Neumann, G., 2006. Localized sampling of root exudates and rhizosphere soil solution by use of sorption media – Applications and Perspectives. The First Joint Conference of the German Society for Plant Nutrition (Annual Meeting), 26.-28.09.2006, Stuttgart, Germany.

Haase, S., Kandeler, E., Kuzyakov, Y., Kania, A., Edelkott, I., Marschner, P., Römheld, V., Neumann, G., 2006. Localized sampling of root exudates and rhizosphere soil solution by use of sorption media. 18th World Congress of Soil Science, 09.-15.07.2006, Philadelphia, USA.

Haase, S., Neumann, G., Kania, A., Rothe, A., Römheld, V., Kandeler, E., 2004. Root exudation and microbial diversity in the rhizosphere of bean (*Phaseolus vulgaris* L.) as affected by atmospheric CO_2 concentration and N-nutritional status. The Third International Nitrogen Conference (INC), 12.-16.10. 2004, Nanjing, China.

Haase, S., Neumann, G., Kania, A., Rothe, A., Römheld, V., Kandeler, E., 2004. Root exudation and microbial diversity in the rhizosphere of bean (*Phaseolus vulgaris* L.) as affected by atmospheric CO_2 concentration and N-nutritional status. International Congress Rhizosphere, – Perspectives and Challenges – A Tribute to Lorenz Hiltner, 12.-17.09.2004, München, Germany.

Haase, S., Neumann, G., Kania, A., Rothe, A., Römheld, V., Kandeler, E., 2004. Effects of elevated atmospheric CO_2 concentration on root exudation and microbial diversity in the rhizosphere of barley and bean. Eurosoil, 04.-12.09.2004, Freiburg, Germany.

Oral Presentations

Ruess, L., Poll, J., Marhan, S., Haase, S., Kandeler, E., 2007. Linking belowground multitrophic interactions between plants, root herbivores and microorganisms to aboveground plant performance. Annual Meeting of the Society of Ecology , 10.-14.09.2007, Marburg, Germany.

Haase, S., Marhan, S., Neumann, G., Ruess, L., Kandeler, E., 2007. Einfluss des Nematoden *Meloidogyne incognita* auf Wurzelmorphologie, Rhizodeposition und Pflanzen-Mikroben Interaktion bei *Hordeum vulgare*. Annual Meeting of the German Society of Soil Science, 03.-7.09.2007, Dresden, Germany.

Haase, S., 2007. Rhizodeposition und biotische Interaktionen in der Rhizosphäre von Bohne und Gerste. Colloquium of the Institute of Soil Science and Land Evaluation, 02.07.2007 University of Hohenheim, Germany.

Haase, S., Marhan, S., Neumann, G., Ruess, L., Kandeler, E., 2006. Einfluss des Nematoden *Meolidogyne incognita* auf Wurzelmorphologie und Pflanzen-Mikroben Interaktionen bei *Hordeum vulgare*. Symposium „Ökophysiologie des Wurzelraumes“ 25.-26.09.2006, Hohenheim, Germany.

Marhan, S., Poll, C., Haase, S., Bisharat, R., Erbs, M., Fangmeier, A., Kandeler, E., 2006. Einfluss von erhöhter atmosphärischer CO_2-Konzentration auf die mikrobielle Besiedlung und den Abbau zweier Streuarten (Sommerweizen und Kornblume) in einem Ackerboden. Meeting of Commission III (Soil Biology) and Comission VIII (Soil Protection) of the German Society of Soil Science, Braunschweig, Germany.

Haase, S., Neumann, G., Kuzyakov, Y., Römheld, V., Kandeler, E., 2005. Einfluss von erhöhter atmosphärischer CO_2-Konzentration auf Wurzelexsudation und mikrobielle Diversität in der Rhizosphäre von Bohne (*Phaseolus vulgaris* L.) in Abhängigkeit der Stickstoffernährung. Annual Meeting of the German Society of Soil Science, 03.-11.09.2005, Marburg, Germany.

Haase, S., Neumann, G., Kania, A., Rothe, A., Römheld, V., Kandeler, E., 2004. Root exudation and microbial diversity in the rhizosphere of bean (*Phaseolus vulgaris*) as affected by atmospheric CO_2 concentration and N-nutritional status. Symposium: Sustainable Agriculture and Land Use Strategies; 7.-8.10.2004; Peking, China

Krumrei, S., Feger, K.H., Lorenz, K., Preston, C.M., 2003. Dynamik des Massenverlustes sowie der Elementfreisetzung bei der Zersetzung von Blatt- bzw. Nadelstreu von Kiefer, Buche, Eiche und Traubenkirsche auf einem armen Standort in der nördlichen Oberrheinebene. Annual Meeting of the German Society of Soil Science, 03.08.-07.09.2003, Frankfurt/Oder, Germany.

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Haase, S., Rothe, A., Kania, A., Wasaki, J., Römheld, V., Engels, V., Kandeler, E., Neumann, G., 2008. Response to iron limitation in *Hordeum vulgare* L. as affected by atmospheric CO_2 concentration. Journal of Environmental Quality, accepted.

Haase, S., Philippot, L., Neumann, G., Marhan, S., Kandeler, E., 2008. Local response of bacterial densities and enzyme activities to elevated atmospheric CO_2 and different N supply in the rhizosphere of *Phaseolus vulgaris* L. Soil Biology & Biochemistry, in press DOI: 10.1016/j.-soilbio.2007.12.025.

Wasaki, J., Kojima, S., Maruyama, H., Haase, S., Osaki, M., Kandeler, E., 2008. Localization of acid phosphatase activities in the roots of white lupin plants grown under phosphorus-deficient conditions. Soil Science and Plant Nutrition 54, 95-102.

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 rhizodepostion in host plants (*Hordeum vulgare*). Plant and Soil 301, 151-164.

Poll, J., Marhan, S., Haase, S., Hallmann, J., Kandeler, E., Ruess, L., 2007. Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. FEMS Microbiology Ecology 62, 268-279.

Haase, S., Neumann, G., Kania, A., Kuzyakov, Y., Römheld V., Kandeler, E., 2007. Elevation of atmospheric CO_2 and N-nutritional status modify nodulation, nodule carbon supply and root exudation of *Phaseolus vulgaris* L. Soil Biology & Biochemistry 39, 2208-2221.

Lorenz, K., Preston C.M., Krumrei, S., Feger, K.H., 2004. Decomposition of needle/leaf litter from Scots pine, black cherry, common oak and European beech at a conurbation forest site. European Journal of Forest Research 123, 177-188.

Other Publications:

Kandeler, E., Haase S., Deiglmayr K., Ruess L., Marhan, S., Neumann, G., Römheld, V., Bonkowski, M., Philippot L., 2005. Small-scale distribution of soil microbial communities and enzyme processes in the rhizosphere. In: International Biomicrocosmos Workshop- for the Enhancement of Rhizosphere Research. Creative Research Initiative Sousei (CRIS), Hokkaido University, Sapporo (extended abstract).

Krumrei, S., Feger, K.H., Lorenz, K., Preston, C.M., 2004. Dynamik des Massenverlustes sowie der Elementfreisetzung bei der Zersetzung von Blatt- bzw. Nadelstreu von Kiefer, Buche, Eiche und Traubenkirsche auf einem armen Standort in der nördlichen Oberrheinebene. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 103, 147-148.

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