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How does nitrogen availability alter rhizodeposition in *Lolium multiflorum* Lam. during vegetative growth?

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Abstract

The objective of this work was to determine if the impact of nitrogen (N) on the release of organic carbon (C) into the soil by roots (rhizodeposition) correlated with the effect of this nutrient on some variables of plant growth. *Lolium multiflorum* Lam. was grown at two levels of N supply, either in sterile sand percolated with nutrient solution or in non-sterile soil. The axenic sand systems allowed continuous quantification of rhizodeposition and accurate analysis of root morphology whilst the soil microcosms allowed the study of ^{14}C labelled C flows in physico-chemical and biological conditions relevant to natural soils. In the axenic sand cultures, enhanced N supply strongly increased the plant biomass, the plant N content and the shoot to root ratio. N supply altered the root morphology by increasing the root surface area and the density of apices, both being significantly positively correlated with the rate of organic C release by plant roots before sampling. This observation is consistent with the production of mucilage by root tips and with mechanisms of root exudation reported previously in the literature, i.e. the passive diffusion of roots solutes along the root with increased rate behind the root apex. We proposed a model of root net exudation, based on the number of root apices and on root soluble C that explained 60% of the variability in the rate of C release from roots at harvest. The effects of N on plant growth were less marked in soil, probably related to the relatively high supply of N from non-fertiliser soil-sources. N fertilization increased the shoot N concentration of the plants and the shoot to root ratio. Increased N supply decreased the partitioning of ^{14}C to roots. In parallel, N fertilisation increased the root soluble ^{14}C and the ^{14}C recovered in the soil per unit of root biomass, suggesting a stimulation of root exudation by N supply. However, due to the high concentration of N in our unfertilised plants, this stimulation was assumed to be very weak because no significant effect of N was observed on the microbial C and on the bacterial abundance in the rhizosphere. Considering the difficulties in evaluating rhizodeposition in non sterile soil, it is suggested that the root soluble C, the root surface area and the root apex density are additional relevant variables that should be useful to measure along with the variables that are commonly determined when investigating how plant functioning impacts on the release of C by roots (i.e soil C, C of the microbial biomass, rhizosphere respiration).

Introduction

In the environment surrounding roots, the rhizosphere, the release of organic carbon (C) from roots (rhizodeposition) (Shamoot et al., 1968), increases not only the number of microorganisms, but also their activity (Nguyen and Henry, 2002; Söderberg and Baath, 1998). The stimulation of microbial activity by rhizodeposition is assumed to impact on nitrogen (N) dynamics (mineralisation and microbial immobilization) in the rhizosphere (Kuzyakov, 2002; Norton and Firestone, 1996; Reydellet et al., 1997; Whalen et al., 2001) and consequently on the availability of N to the

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plant (Hodge et al., 2000; Kaye and Hart, 1997). Besides, the literature indicates that N fertilization can impact on rhizodeposition but no simple relationship between N availability and rhizodeposition can be derived. In sterile nutrient solution, Paterson and Sim (1999; 2000) did not find differences between the total C released by *Festuca rubra* or *Lolium perenne* in response to high and low N supply. Using the pulse ^{14}C-labelling of photoassimilates, Johansson (1992) found that the quantity of ^{14}C released into the soil by barley roots was not significantly affected by N fertilisation, whereas Liljeroth et al. (1994) with maize and Zagal et al. (1994) with barley, found that N fertilisation increased the rhizodeposition of ^{14}C. Besides the fact that these studies concerned different plant species and different experimental conditions, these contradictory results can be reconciled by consideration of the multiplicity of the mechanisms that control rhizodeposition and that are differently affected by N availability.

The release of recently fixed C from roots depends on C exportation from shoots to belowground (Swinnen et al., 1994a; 1994b), which is decreased by increased N availability (Johansson, 1992; Liljeroth et al., 1994; Paterson and Sim, 1999). A major component of the rhizodeposition of recently fixed C is exudation, which is the passive diffusion of low molecular weight solutes from the cytosol to the soil solution. Exudation takes place along the root surface, commonly with higher rates at the root tips and sites of lateral root emergence (McDougall and Rovira, 1970 Jones, 1998; Darwent et al., 2003;). Therefore, the magnitude of exudation is expected to be dependent on the root morphology, which, in turn, is highly related to N availability. Indeed, the increase in N availability generally reduces root length (Liljeroth et al., 1990; Xu and Juma, 1993), and increases root branching and consequently the number of root apices (Bloom, 1997). Furthermore, the increase in branching frequency correlates well with an increase in the concentration of sugars in roots (Freixes et al., 2002). This likely favours exudation by increasing the C gradient between the root and the soil. This gradient is also increased and maintained by the microbial growth in the rhizosphere, which is expected to be stimulated by N fertilisation because increasing N availability reduces the competition for N between the roots and the microorganisms (Van Veen et al., 1989). Consistently, the bacterial abundance and activity in the rhizosphere have been reported to be greater in plants fertilized with N (Christensen and Christensen, 1994; Liljeroth et al., 1990).

This overview of the literature illustrates that there are many potential mechanisms by which N can increase or decrease the release of C by roots. As these results were obtained from various experimental systems and for different plant species, it is difficult to determine the mechanisms responsible for effects of N supply on rhizodeposition. Therefore, this work aimed to evaluate on a given plant, *Lolium multiflorum* Lam. the different mechanisms by which N alters rhizodeposition. For this, we examined how two levels of N fertilization affected rhizodeposition, and to what extent changes in rhizodeposition correlated with shifts in biomass accumulation and partitioning, with root morphology and with soluble C concentrations in root tissues.

A first experiment was performed on plants grown in sterile percolated sand systems because it allowed a direct quantification of the root released C, which is not possible in soil because rhizodeposits are rapidly mineralised by microorganisms (Meharg, 1994; Todorovic et al., 2001). Furthermore, sand cultures facilitate the harvest of the roots and the study of their morphology. Besides, plants grown in non sterile soil at two levels of N were also studied in a second experiment to take into account the interactions between the roots, the soil matrix and the microflora (Kuzyakov and Domanski, 2000). These plants were used for the pulse labelling of photo-assimilates with ^{14}C to investigate the pattern of current assimilate partitioning between plant compartments as well as between the plant and the soil C pools (Meharg, 1994; Swinnen et al., 1994a, b). Since rhizodeposition cannot be quantified in non-sterile soil, we used indirect techniques indicating the availability of C in the rhizosphere of plants at the two levels of N applied (the bacterial abundance and the microbial C in the rhizosphere).

Materials and methods

Sterile percolated sand cultures

Seeds of *Lolium multiflorum* Lam. were surface sterilized in 0.5% (v/v) peracetic acid for 20 min as described by Paterson and Sim (1999, 2000). The sterilized seeds were transferred aseptically onto glass Petri dishes containing sterile moist sand and they were allowed to germinate in the dark at 20 °C. After

root emergence (4 days later), the seeds were transferred to a light bench (16 h day length at 450 μmol $m^{-2}\,s^{-1}$, 20 °C) for 4 days prior to planting into the microcosms. A sub-sample of the sterilized seeds was placed onto malt agar, and incubated for 8 days to confirm sterility of seeds.

The microcosm design used, was originally described by Hodge et al. (1996) and modified by Paterson and Sim (1999, 2000). The system was designed to measure net exudation from roots. Net exudation is the balance between passive, diffusive release of root metabolites and active re-uptake of these compounds (Jones and Darrah, 1993). The continuous percolation of nutrient solution through the sand matrix provides a means of standardising C-sink strength exterior to roots. Use of ^{14}C-labelled tracers has demonstrated that N-supply and shoot clipping treatments did not affect the extent of re-uptake for *Lolium perenne* (Paterson and Sim, 1999). The assembled microcosms and tubing were autoclaved (121 °C, 20 min) in sealed bags, after first wetting the sand matrix to optimise sand sterilization. Prior to planting, the sand was percolated (45 mL day^{-1}) with sterile demineralised water for 4 days. Then, a 0.25 strength Hoagland's nutrient solution (Hoagland and Arnon, 1950), modified to contain either 4 mM or 0.1 mM NO_3^- as KNO_3 and $CaNO_3$ (1/1) was supplied. The corresponding N-supply rates were 180 and 4.5 μmoles N d^{-1} for high and low N supplies, respectively. These supply rates were selected to provide N in excess of that required for optimal growth (high N) and limiting to growth relative to the balance of other nutrients (low N).

Seedlings (3 per microcosm) were transferred aseptically into the top chamber of the microcosm and planted into the sand. The microcosms were then placed into a controlled environment room (Conviron, Winnipeg, Canada). The environmental conditions were set to give a constant temperature of 20 °C, with a 16 h day length and a PAR of 325 μmol $m^{-2}s^{-1}$ within the chambers. The impact of the glass chamber on internal (plant-experienced) conditions necessitated that during the light period, the PAR at plant level outside the chamber was 700 μmol $m^{-2}s^{-1}$ and the external temperature was set to 6 °C to counter the radient heat generated. Air from the growth room was pumped through the top chamber of each microcosm after passing through a 0.2 μm filter to maintain sterility; flow rate was adjusted to 60–80 mL min^{-1}.

Nutrient solution that had percolated through the sand matrix was collected every 3–4 days by generating negative pressure in an acid washed sterile glass collection vessel connected to the bottom chamber. Sterility of the microcosms was maintained by using a 3-way Luer-lock, which isolated the microcosm, except during solution collection. Sterility of the collected leachate was checked by plating directly onto LB agar. Low levels of contamination ($<$1000 cfu mL^{-1}) were detected in all collected samples. This contamination most likely originated from the exposed Luer-lock connection, as no evidence of microbial contamination of the sand matrix was found on harvest. Following leachate collection, solution volume was determined by weight and an aliquot was dispensed for pH measurement. The remainder was stored at -18 °C prior to analysis for total organic C.

As a result of the high growth rate of plants in the microcosms, all the plants had to be cut aseptically at 4 cm height after 19 days of growth to avoid any limitation due to the size of the culture system. The shoot cutting was done within a laminar flow hood, while maintaining flow of sterile air through the shoot chamber. The shoots were cut with sterile scissors and removed with sterile forceps (Paterson and Sim, 1999). The cutting was done 9 d prior to harvest of the plants, allowing sufficient time for recovery of the plants (De Visser et al., 1997; Paterson et al., 2003).

Plants were sampled after 4 and 5 weeks of growth for the high N treatment and the low N treatment, respectively. The top chambers were removed and the plants were excised at the sand surface. The shoots were then freeze-dried. The roots with adhering sand were washed with nutrient solution to avoid any osmotic shock and weighed. Ten percent of the fresh weight of the root system were subsampled, weighed and kept for morphological analysis (LA 1600+, WhinRHIZO, Regent Instruments Inc., Québec, Canada). The remaining roots were freeze-dried. Shoots and roots were ground and analysed for their organic C and N content by a C-N micro-analyser (model Flash EA1112, Thermo Finnigan, France). The soluble C in the roots was determined from an aliquot of 100 mg of dry milled root that was extracted with 5 mL of 80% ethanol at 80 °C for 20 min. After centrifugation at 10 000 *g* for 10 min, the supernatant was collected and the pellet re-extracted twice. The three fractions of ethanol were pooled and evaporated under vacuum. The residues were then solubilised in 15 mL of ultra-pure water and analysed for the organic C content by a TOC analyser (model TOC-V CSH/CSN, Shimadzu, Japan).

The soluble C in the percolated nutrient solution was analysed by a TOC analyser. The final daily root exudation was calculated as the average rate of C released from roots per day during the last three days before sampling. There were initially 8 replicates by N level but due to poor establishment after transplanting seedlings into the microcosms, only 4 replicates could be used in the low N treatment.

Planted soil cores

Three seeds were planted into PVC cylinders (diameter 7 cm, length 25 cm) filled with 1450 g dry weight of a mixture of 1/3 river sand and of 2/3 soil. An agricultural soil collected (0-30 cm depth) at the INRA experimental station at Mirecourt (Vosges, France) was used (clay: 19.1%; silt: 46.6%; sand: 34.3%; $pH_{H2O} = 6.5$; C: 1.3%; N: 0.13%, N-NO_3^-: 6.9 μg g^{-1} soil, N-NH_4^+: 2.3 μg g^{-1} soil). The dry soil was sieved to 5 mm, rewetted and mixed with 2% (W/W) of wet soil stored at 4 °C following collection from the field. The addition of soil that had not been dried was assumed to limit the effects of drying the soil on the diversity of the microbial communities. The soil was then incubated for 4 weeks in the dark at room temperature. After germination, seedlings were removed to leave one plant per cylinder. The plants were grown for five weeks with a 14 h photoperiod, at 23 °C, under 75% of relative humidity and 350 μmol m^{-2} s^{-1} of photon flux density in the PAR. The soil water content was maintained at 70% of its water holding capacity by daily weighing and addition of distilled water. After one week of growth, half of the cylinders were fertilized with 230 mg/cylinder of ammonium nitrate, equivalent to 150 kg of N ha^{-1} (5 plants per N treatment).

Five-week-old plants, fertilized or not, were used for the labelling of photo-assimilates with $^{14}CO_2$. Shoots were separated from the root/soil compartment as described by Nguyen et al. (1999). Briefly, the soil cores were placed into polypropylene tubes whose upper screw cap contained a central hole for the shoots and a hole for the supply of water. Two other holes were used as inlet/outlets for flushing of the below-ground compartment with CO_2-free air and trapping of respiration by bubbling into 1 M NaOH. Liquid silicon was poured into a central plastic tube, surrounding the pseudo-stem. The plants were placed inside a transparent chamber itself installed within a growth cabinet to maintain the aforementioned environmental conditions. Then, plants were exposed for two hours to a $^{14}CO_2$-enriched atmosphere whose ^{14}C-specific activity and CO_2 concentration were regulated at 1920 KBq mmol^{-1} and 350 ppm, respectively. The total ^{14}C assimilated by the ten plants was 22.47 MBq. At the end of labelling, the atmosphere of the chamber was flushed with ambient air and $^{14}CO_2$ was trapped with soda lime. Two days after labelling (48 h), the soil cores were sampled. Plants were gently shaken to separate the soil not adhering to roots. Shoots were cut at the soil level and stored at −20 °C. All roots were carefully removed by hand from the soil, which was then weighed. The soil adhering to roots was collected by washing the roots with a solution of 1 mM $CaCl_2$ (Neumann and Römheld, 2000). The $CaCl_2$ slurry was then centrifuged at 6000 *g*. The soil pellet (adhering soil) was stored at 4 °C before analysis (within 3 days). The supernatant was stored at −20 °C.

Freeze-dried shoots and roots were milled to a fine powder and analysed for their total C and N content by a C-N micro-analyser. The ^{14}C content of the plant material was determined by a flow radioactivity detector coupled on-line to the micro-analyser (Benoit et al., 1994). For soil samples, which had lower ^{14}C activity, the flow radioactivity detector did not give reliable determination of the sample radioactivity. Therefore, the ^{14}C activity of soils was determined separately by dry combustion of larger sub-samples (ca. 1–2 g dry weight) (Todorovic et al., 1999). The soluble C of the roots was extracted by hot ethanol and quantified as for axenic plants. The root soluble ^{14}C activity was determined by liquid scintillation counting using Ultima Gold scintillation cocktail (Packard, USA). The ^{14}C in the NaOH traps was counted by liquid scintillation using Ultima Gold scintillation cocktail.

Total C in the microbial biomass was determined by the fumigation/extraction method (Vance et al., 1987) using a K_{ec} of 0.45 (Wu et al., 1990). Nitrate and ammonia content of soil were determined by colorimetry (TRAACS 2000, Bran-Luebbe) from the $CaCl_2$ solutions and from the K_2SO_4 extracts of the non fumigated soils.

The number of cultivable bacteria in the adhering soils was determined from 10 g of the pellet obtained after centrifugation of the $CaCl_2$ slurry. These soil aliquots were extracted for 10 min in 100 mL PBS buffer. The soil slurry was centrifuged at 500 *g* for 15 min and the supernatant was diluted in series up to 10^{-6}. A 100 μL aliquot of 10^{-6} and 10^{-5} dilutions

Table 1. Dry matter (DM) production, C and N content, root morphology and net root exudation of *Lolium multiflorum* grown in axenic sand culture under high and low levels of N, for 28 and 35 days, respectively. Standard deviation is given in parenthesis

	High N ($n = 8$)		Low N ($n = 4$)		P value	High N/ Low N
Number of tillers	11	(1.6)	3	(0.0)	***	3.7
Shoot dry weight (g)	0.61	(0.192)	0.10	(0.033)	***	6.1
Root dry weight (g)	0.41	(0.099)	0.62	(0.120)	*	0.7
Shoot C to root C ratio	2.1	(0.97)	0.4	(0.24)	**	5.6
Shoot N (% shoot DM)	5.4	(0.80)	1.0	(0.14)	***	5.4
Root N (% root DM)	1.7	(0.28)	0.3	(0.13)	***	5.7
Root soluble C (% root DM)	2.4	(0.50)	2.9	(1.02)	ns	0.8
Number of root apices	953	(413.6)	424	(74.7)	*	2.2
Root length (cm)	1316	(531)	840	(164)	ns	1.6
Root surface area (cm^2)	193.5	(49.10)	124.9	(18.18)	*	1.5
Total C exudation (mg)	4.16	(0.645)	2.99	(1.291)	(*)	1.4
Average of daily root exudation during the three days before sampling						
mg C day^{-1} $microcosm^{-1}$	0.13	(0.05)	0.05	(0.031)	**	2.8
% root C day^{-1}	0.12	(0.051)	0.04	(0.020)	**	3.1

The P value associated to ANOVA Fischer test is presented as follows: ns: $p \geq 0.1$, (*): $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

were plated on 10% TSA agar and incubated at 27 °C in the dark. The colonies formed were counted at 48 h.

Statistics

Statistical analyses were performed with SAS software (SAS for Windows V 8.02, The SAS Institute Inc., Cary, NC). The effect of N fertilization was tested using the general linear model (proc GLM) because of unequal number of replicates between the treatments. The validity of the assumptions related to the general linear model was evaluated on residuals by the Kolmogorov–Smirnov test for normality, by a residuals versus predicted plot for appreciating homosedasticity and outliers. When necessary, data were $\log_{10}$ transformed. Correlation coefficients were computed with the procedure CORR (correlation). Proc MODEL was used for estimating parameters used in the non linear equation of the exudation model.

Results

Sterile sand cultures

Biomass of high N plants was significantly larger than that of low N plants (1.02 g and 0.72 g of dry matter, respectively, $p = 0.007$). The plants grown at high N had four times more tillers than plants grown at low N (Table 1). Increased N supply significantly favoured shoot growth since the dry weight of shoots was six times higher in high N plants than in the low N treatment. The root dry weight was significantly lower in high N plants compared to low N plants but this might be related to the difference in length of the cultivation between the two treatments (low N plants were one week older than high N plants). Plants supplied with high N were 5 times richer in N than low N plants. The shoot C to root C ratio was six times greater for high N plants compared to low N. The soluble C in roots was unchanged by the N level. The number of root apices was increased by N. No significant differences in root length were evidenced between the two treatments. Consequently, the mean apex density per unit of root length was greater in high N plants. The N fertilization increased significantly the root surface area. All of these root morphological variables were correlated to each other (data not shown).

The total amount of C released from roots (net exudation) over the respective growing periods was increased in high N plants ($p = 0.056$) compared to low N plants, despite the longer growing period for low N plants (Table 1). In addition, the final rate of net exudation during the three days before the sampling was three times larger in high N plants. Moreover, this

Table 2. Net exudation of *Lolium multiflorum* grown in axenic sand cultures at two levels of N. Models fitted to the average of daily root exudation during the three days before sampling (E) in relation to root variables (S_r: root surface area (cm^2); C_r: root soluble C expressed as percentage of root dry weight; Apex: number of apices)

			R^2	D_1 (mg C cm^{-2} day^{-1}) D_2 (mg C $apex^{-1}$ day^{-1})		
				Estimate	Approx. S.E.[a]	Approx. $p > \|t\|$[b]
Model 1 $E = D_1.S_r.C_r$	High N	($n = 8$)	0.54	$2.5\ 10^{-4}$	$0.26\ 10^{-4}$	***
	Low N	($n = 4$)	0.28	$1.7\ 10^{-4}$	$0.58\ 10^{-4}$	***
	High + Low N	($n = 12$)	0.51	$2.2\ 10^{-4}$	$0.27\ 10^{-4}$	***
Model 2 $E = D_2.Apex.C_r$	High N	($n = 8$)	0.25	$4.6\ 10^{-5}$	$0.61\ 10^{-5}$	***
	Low N	($n = 4$)	0.35	$4.1\ 10^{-5}$	$1.00\ 10^{-5}$	*
	High + Low N	($n = 12$)	0.60	$4.5\ 10^{-5}$	$0.49\ 10^{-5}$	***

[a]Approximate standard error.
[b]Probability associated to the null hypothesis, presented as follows: ns: $p \geq 0.1$, (*): $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

stimulating effect of N was also observed if root exudation rate was expressed as a percentage of the root C (Table 1).

Given that exudation is driven by the passive diffusion of root soluble C to the surrounding environment (Grayston et al., 1996; Jones, 1998), it can be described by Fick's first law:

$$E = S_r{}^*D^*(C_{root} - C_{ext}), \quad (1)$$

where E (mg of C day^{-1} $microcosm^{-1}$) is the exudation rate, S_r (cm^2) is the root surface area, D (L cm^{-2} day^{-1} $microcosm^{-1}$) is the diffusion coefficient of solutes through the root tissues, C_{root} (mg of C L^{-1}) is the soluble C concentration within the root and C_{ext} (mg of C L^{-1}) is the C concentration in the external solution. We assumed that root exudation was in a steady state and consequently that $C_{ext} = K^*C_{root}$ (K = constant). Hence, (1) can be re-arranged to:

$$E = S_r{}^*D_1{}^*C_r \qquad \text{(Model 1)},$$

where C_r is the root soluble C expressed as percentage of root dry matter and D_1 is a constant. C_r was used rather than C_{root} (mg of C L^{-1}) because of the inaccuracy in the determination of the water content of root tissues. Since exudation is assumed to be more important at root apices, the following model was also evaluated:

$$E = D_2{}^*Apex^*C_r \qquad \text{(Model 2)},$$

where Apex is the number of root apices.

The models were fitted to experimental data from high and low N treatments. The D_1 and D_2 estimates for the low N treatment were similar to D_1 and D_2 estimates for high N treatment, respectively (Table 2). Therefore, the models were also fitted to data pooled from both N treatments. More than 50% of the variability in the final daily root exudation were explained by model 1 ($R^2 = 0.51$; Table 2). If the root surface area was replaced by the number of branches (model 2; Table 2), the model explained 60% of the variability in the final daily root exudation and the D_2 estimate was highly significant ($p < 0.001$). A model considering both the root surface area and the number of root apices was also evaluated (results not presented) but it gave non significant D_1 and D_2 estimates because of the strong correlation between the root surface area and the number of apices ($R^2 = 0.88$; $p = 0.001$; data not shown).

Planted soil cores

The effect of N fertilisation on biomass production was moderate (Table 3). N supply increased the shoot dry weight ($p < 0.1$) and the shoot C to root C ratio ($p < 0.05$) but had no significant effect on the root dry matter. Fertilised plants were richer in N. This was highly significant for shoots ($p < 0.01$) but not for roots ($p < 0.1$). We observed that the soluble C concentration of the roots was significantly higher in fertilized plants. In the soil adhering to roots, the nitrate concentration was significantly higher in high N treatment compared to low N treatment (Table 4) for which nitrate concentration was around the initial level (6.9 μg g^{-1} soil). Ammonium concentration in adhering soil was not changed by N fertilisation (data not shown). Microbial C was around 190 μg C g^{-1} soil and was not affected by N fertilization. Microbial

Table 3. Means of dry matter (DM), shoot C to root C ratio, concentration in N and root soluble C of *Lolium multiflorum* grown in soil under two levels of N ($n = 5$). Standard deviation is given in parenthesis

	High N		Low N		*P* value	High N/ Low N
Shoot DM (g)	1.7	(0.52)	1.2	(0.23)	(*)	1.4
Root DM (g)	1.0	(0.66)	2.0	(1.37)	ns	0.5
Shoot C : Root C ratio	4.8	(0.82)	3.1	(1.04)	*	1.5
Shoot N (% DM)	4.7	(0.69)	3.4	(0.33)	**	1.4
Root N (% DM)	0.9	(0.27)	0.6	(0.34)	(*)	1.5
Root soluble C (% root DM)	1.43	(0.56)	0.69	(0.40)	*	2

The P value associated to ANOVA Fischer test is presented as follows: ns: $p \geq 0.1$, (*): $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$.

abundance (cultivable bacteria) was also unchanged by N supply.

The total ^{14}C recovered in the plants and soil after sampling was 19.07 MBq (data not shown). Given that the ^{14}C assimilated by plants during the labelling was 22.47 MBq, the recovery yielded 84.9%. The total ^{14}C recovered/plant was not significantly different between the fertilized and the non fertilized plants ($p = 0.214$). As all plants were labelled together within a single chamber, it was not possible to determine the balance between net and gross assimilation of ^{14}C for individuals. The N fertilization decreased the ^{14}C recovered in roots (11.5% of the ^{14}C recovered in high N versus 20.1% in low N, $p = 0.008$). The ^{14}C recovered in shoots, in rhizosphere respiration and in soil were unchanged by N supply (results not presented). However, relative to the root dry matter, N supply increased the ^{14}C recovered in the rhizosphere respiration (root + microbial respiration) and in the soil residues (Table 4). In addition, the ^{14}C activity of the root soluble C was higher in N fertilized plants (Table 4) and we observed that the ^{14}C in soil g^{-1} root was strongly and highly positively correlated to the root soluble ^{14}C concentration ($R^2 = 0.88$, $p < 0.001$).

Discussion

Effect of N on plant growth and partitioning of photo-assimilates

This study was undertaken to examine how changes in plant growth in response to N fertilization affect the release of C from roots. Therefore, the prerequisite was a stimulating effect of N on plant growth. This was clearly observed for axenic sand cultures, for which the high level of N strongly stimulated the overall plant growth and more particularly that of the shoots and the number of tillers (Table 1). Although the growth period for plants supplied with low N was longer than for high N plants, on harvest, the high N plants had a higher biomass. Therefore, it is unlikely that the longer growth period of low N plants confounded the results as a consequence of ontogenic effects. Indeed, since shoot:root ratio generally increases with plant age (Gedroc et al., 1996) and was found to be less for plants grown for longer (low N plants), the effects on partitioning were attributed primarily to the relative N-supply rates. In addition the N concentration of the tissues was five times larger in the high N plants compared to low N plants. These effects (increase of plant biomass, of shoot:root ratio and of N content of tissues) are typical of a stimulation of plant growth by N (Johansson, 1992; Liljeroth et al., 1994; Marschner et al., 1996; Mikan et al., 2000; Paterson and Sim, 1999; 2000; Zagal et al., 1994).

In percolated sand cultures, N increased the number of apices and the total root surface area (Table 1). Here again, it is unlikely that the difference in plant age between the two N treatments (one week) was responsible for the effect on the root morphology. Indeed, the difference in root morphology between the two treatments was consistent with the strategy of roots growing in a nutrient rich environment, which is characterised by increasing the absorptive surface area of roots rather than increasing the volume of soil exploited (Fitter, 1991).

In planted soil cores, the effect of N on plant growth was much less marked (Table 3). Indeed, N supply poorly stimulated shoot biomass. However, N fertilisation increased the N content of shoots and

Table 4. Means of nitrate concentration, microbial C, bacterial density (Log CFU) and belowground ^{14}C relative to root dry matter (DM) in rhizosphere soil of *Lolium multiflorum* grown under two levels of N ($n = 5$). Standard deviation is given in parenthesis

	High N		Low N		*P* value	High N/ Low N
Nitrate (μg N g^{-1}soil)	81.0	(26.14)	5.6	(8.71)	***	14.5
Microbial Biomass C (μg g^{-1}soil)	196.9	(80.1)	181.3	(146.95)	ns	1.1
Log (CFU g^{-1} soil)	6.0	(0.23)	6.2	(0.29)	ns	1.0
Rhizosphere $^{14}CO_2$ (KBq g^{-1} root DM)	377.5	(178.27)	144.3	(86.93)	*	2.6
Soil ^{14}C (KBq g^{-1} root DM)	103.6	(46.18)	51.7	(32.58)	*	2.0
Root soluble ^{14}C (KBq g^{-1} root DM)	71.4	(43.20)	15.9	(12.14)	*	4.5

The *P* value associated to ANOVA Fischer test is presented as follows: ns: $p \geq 0.1$, (*): $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

favoured above ground growth relative to roots as illustrated by the shoot C: root C ratio and by the lower allocation of ^{14}C to roots in N fertilized plants. It is unlikely that the amount of N supplied to soil cultures was too low to stimulate plant growth because the rate of N applied was equivalent to a typical fertilizer application (150 kg N ha^{-1}) and the N concentration of shoots in N fertilized soil was equivalent to that of high N plants grown in sand. However, the N concentration of tissues in control plants grown in soil (3.4%) was not as low as expected. Therefore, it is assumed that the supply of N from the soil was sufficiently high to largely negate the expected positive effects of N fertilisation on plant growth.

In the planted soil cores, both the ^{14}C and the non-radioactive soluble C concentration in roots were increased by N fertilization and might be related to increased specific costs of root activity in fertilized plants associated with the uptake and reduction of N. The lack of difference in the root soluble C between high and low N plants cultivated in sand was unexpected since the effects of N on plant growth were particularly marked. In soil conditions, the established stimulation of root exudation by microorganisms (Barber and Martin, 1976; Lee and Gaskins, 1982) may directly deplete root soluble C-concentrations. Therefore, where a treatment affects the size of the microbial sink, it may also influence soluble root C concentrations. However, as we found no difference in microbial C and in bacterial abundance between high N and low N soil-grown plants, it is likely that differential energetic costs of N-acquisition were responsible for treatment differences in root soluble C for these plants. For the axenic systems, the sink strength outside the roots is determined by the flow rate of nutrient solution, which in this study was constant between treatments. This could explain the lack of effect of N-treatment on root soluble C-concentrations for the axenically grown plants.

Relationships between rhizodeposition and the effect of N fertilization on plant growth

It is reasonable to assume that the major rhizodeposit classes quantified in this study were exudates, mucilages and to a lesser extent border cells. Indeed, senescing root tissues were unlikely to have been significantly labelled by the pulse of ^{14}C (Meharg, 1994), and in percolated cultures the nutrient solution analysed for organic C did not contain any visible root debris. Given that (1) exudation is assumed to be quantitatively more important than the release of mucilage and border cells (Nguyen, 2003), and that (2) exudation is driven by the C gradient between the root tissue and the external solution, the amounts of organic C released from roots in axenic conditions were fitted to models derived from the diffusion law. The models explained more than 50% of the variability in the net release of C from roots in axenic culture. Moreover, when the number of apices was used instead of the root surface area in the model, the goodness of fit was increased by nearly 10% ($R^2 = 0.60$; Table 2). It outlines that, in our work, the number of branches was an important variable controlling exudation, consistently with the fact that exudation is higher at root apices and at the sites of emergence of lateral branches (Darwent et al., 2003; McDougall and Rovira, 1970).

It may also evidence the production by root apices of mucilages and border cells that were not distinguished from the exudates *sensu stricto* (i.e. root solutes diffusing through the root tissues). Whichever model was applied, the parameter estimates obtained for the high N treatment were equal to those obtained for low N treatment. This suggests that the release of root solutes relative to the number of root branches and to the concentration of soluble C in root tissues was likely not affected by the N supply nor by the one-week difference in plant age between the two N levels.

It is important to note that exudation quantified in the axenic cultures is resultant of the balance between gross exudation and re-uptake of exuded compounds from roots (Jones and Darrah, 1993). The axenic system design incorporates continuous percolation of nutrient solution, which standardises the potential for re-uptake and does not suffer time-dependent effects of re-sorption from static solutions (Hodge et al., 1996). However, in soils the extent of re-uptake may vary with N-availability, as a consequence of changing microbial activity and consequently C-sink strength exterior to roots. In addition, our data do not allow separate evaluation of the effects of N-availability on plant regulation of re-uptake processes. Further work is required to determine effects of N-availability on transporters for re-uptake of specific exudate compounds, as such physiological adaptations could markedly affect the quality of C present in net exudation.

In planted soil cores, the effects of N on C release from roots were not as distinct as they were in percolated sand cultures. Total microbial C, and bacterial abundance were not significantly changed by N fertilization. Relative to the root dry matter, a larger proportion of fixed ^{14}C evolved as rhizosphere respiration from fertilised plants (Table 4). However, it cannot be stated with confidence that this resulted from increased microbial mineralisation of ^{14}C-labelled rhizodeposition only. Indeed, it might be attributed to (i) an increased root respiration due to the uptake and reduction of N and to (ii) an increased turnover of microorganisms, which may have been more severely limited by some other nutrient at high N since apart from N, no other minerals were added to the soil. However, the ^{14}C in soil residues per unit of root dry matter was significantly increased by N fertilization (Table 4), suggesting a stimulation of rhizodeposition of recently fixed C. In addition, the ^{14}C in soil residues relative to the root biomass was strongly correlated with the root soluble ^{14}C ($R^2 = 0.88$, $p < 0.001$), which is consistent with the passive diffusion of root solutes to the soil solution (exudation). So, from our point of view, there was likely a stimulation of rhizodeposition by N fertilization in the planted soil cores as suggested by the ^{14}C tracing of recent photoassimilates. However, this stimulation was too minor to be evidenced by indirect measurements (e.g. bacterial abundance, microbial C). The weak effect of N fertilisation on rhizodeposition is consistent with the poor response of the plant to the added N. There was likely a high supply of plant available N from soil (in the absence of fertiliser addition), supporting the maintenance of high tissue N concentrations in unfertilised plants. Indeed, in percolated sand cultures the five-fold increase in shoot N due to N supply was accompanied by a stimulation of exudation by a factor 2.5. Therefore, in soil conditions, the 1.4 times increase in shoot N might have produced an increase in rhizodeposition that was not detectable by the measurement of microbial C and bacterial abundance.

In conclusion, our work contributed to the identification of some important variables that mediate the effects of N on the release of recently fixed C by roots: the root surface area, the soluble C concentration in root tissues, and the number of root apices. Generally, any factor that alters these variables is thus expected to impact rhizodeposition. Therefore, in soil conditions, which do not allow a direct quantification of rhizodeposition, the determination of the root soluble C, of the number of root apices and of the root surface area are useful parameters from which to infer impacts on root exudation.

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