Functional Ecology 2002
16, 198–203

Root life spans of four grass species from habitats differing in nutrient availability

T. A. J. VAN DER KRIFT and F. BERENDSE†

Nature Conservation and Plant Ecology Group, Wageningen University, Bornsesteeg 69, 6708 PD, Wageningen, The Netherlands

Summary

1. In grass species that occur in pastures or hay meadows, life spans of roots determine much of the carbon and nutrient loss from the plant in addition to the amounts that are lost by mowing or grazing. We hypothesized that grass species from nutrient-poor habitats had longer root life spans and consequently lost smaller quantities of nutrients through root turnover.

2. In a garden experiment, root life spans and root diameters were measured by repeated observations in minirhizotrons placed in monocultures of *Lolium perenne* L. and *Arrhenatherum elatius* L. (characteristic of fertile soils) and *Molinia caerulea* L. and *Nardus stricta* L. (preferring nutrient-poor soils).

3. Average root life spans were 14 weeks in *L. perenne*, 40 weeks in *A. elatius*, 53 weeks in *M. caerulea* and 58 weeks in *N. stricta*. Root life spans of species from fertile habitats were significantly shorter than the root life spans of species from low fertility habitats.

4. In addition, there were significant differences in root diameter among species, root diameter being positively correlated to root life spans. Root diameter decreased during root ageing in all species, while the decline in diameter occurred more slowly in *N. stricta* than in *A. elatius* and *M. caerulea*.

5. An increase in the abundance of plant species adapted to fertile habitats will, because of the greater C and nutrient returns to the soil in root turnover, increase soil fertility. This effect may constitute a positive feedback between changes in plant species composition and nutrient cycling.

Key-words: Minirhizotron, perennial grass species, root diameter, root life span

Functional Ecology (2002) **16**, 198–203

Introduction

The dead organic material produced by plants has an important influence on nitrogen mineralization (Berendse 1990; Bloemhof & Berendse 1995; Van Vuuren *et al*. 1992; Wedin & Tilman 1990). Plants add significant amounts of C and nutrients to the soil through the senescence of their roots. Root production, senescence and decomposition are key processes in the C and N dynamics of ecosystems (Brevedan *et al*. 1996; Van Vuuren *et al*. 1993).

The amount of litter added to the soil, and therefore the importance of biomass turnover for nutrient cycling, differs between species (Aerts *et al*. 1989; Steltzer & Bowman 1998). This has been most clearly demonstrated in studies on plant leaves (Grime 1994; Reich *et al*. 1992). In leaves, a long life span increases nutrient conservation and nutrient-use efficiency. These traits make species successful competitors in nutrient-poor habitats. In nutrient-rich habitats, characteristics associated with short life spans (e.g. small biosynthesis costs and large specific leaf areas) are thought to be important for rapid growth and successful competition (Berendse & Aerts 1987; Chapin 1980; Grime 1994; Poorter & Remkes 1990; Reich *et al*. 1992). These different traits make species potentially successful competitors in either nutrient-poor or nutrient-rich habitats. Root life spans may be linked to suites of traits similar to those found in leaves (Eissenstat & Yanai 1997; Grime 1994). Moreover, the mass and energy involved in the growth and death of roots may match that involved in the growth and death of leaves (Eissenstat & Yanai 1997).

In the study described here, we compared the root life spans of species with a similar growth form, but with a contrasting ecological response to nutrient availability. The species used in this study, *Lolium perenne*, *Arrhenatherum elatius*, *Nardus stricta* and *Molinia caerulea*, are perennial grasses characteristic of soils with different nutrient supplies. *Lolium perenne* and *A. elatius* are fast-growing species typical of nutrient-rich

†Author to whom correspondence should be addressed. E-mail: frank.berendse@staf.ton.wau.nl

habitats, whereas *N. stricta* and *M. caerulea* are characteristic of nutrient-poor habitats. We hypothesized that the roots of the slower-growing species (*N. stricta* and *M. caerulea*) live longer than the roots of the faster-growing species (*L. perenne* and *A. elatius*).

Eissenstat & Yanai (1997) suggest that thin roots have a shorter life span than coarse roots. Therefore we aimed to determine whether there was a link between root life span and diameter. During root ageing, early death of epidermal and cortical cells is an important phenomenon in grasses (Deacon 1987). After the cortex has died, the stele remains alive and presumably still functions in conduction of water and nutrients. In addition, root diameter may decrease because of reabsorption of C and nutrients or shrinkage caused by turgor loss (Gordon & Jackson 2000). To assess biomass and nutrient losses during root senescence preceding complete root death, we measured the change in root diameter during the life span of the roots. Assuming that the roots of species from fertile habitats would have a shorter life span, we hypothesized that their root diameter would decrease more quickly than that of species from less fertile habitats.

Many earlier studies on root life span of grass species were based on core sampling and total root length measurements in minirhizotrons (Schläpfer & Ryser 1996; Troughton 1981). In our study we monitored individual roots by repeated observations in minirhizotrons, which provided more detailed information on the dynamics of the roots that we studied (De Ruijter *et al*. 1996).

Materials and methods

GARDEN EXPERIMENT

The long-term garden experiment started in June 1993. Four different grass species were planted in monocultures arranged randomly within five replicated blocks. Plots of 1 × 1 m were cut out to a depth of 50 cm, at which the yellow sandy subsoil was observed. The plots were subsequently replenished with sandy soil with 6·6% organic matter, 2·0 g kg total N (26·5 mg NO_3^- kg^{-1}, 6·1 mg NH_4^+ kg^{-1}), pH(H_2O) 5·6, that had been sieved to remove old roots. The supplied soil provided a substrate with intermediate soil fertility that was able to sustain populations of both the nutrient-poor and the nutrient-rich grass species. All plots were separated by 50 cm deep underground sheets of waterproof wood surrounding the plots. The plots were planted with monocultures of four grass species, *Lolium perenne* L., *Arrhenatherum elatius* L., *Nardus stricta* L. and *Molinia caerulea* L., chosen because they have a different Clausman N index parameter (Melman *et al*. 1985). This index varies from 1 to 9, and characterizes the relative availability of soil N in the natural habitat where the species in question is most frequently found. Clausman N indices for *L. perenne*, *A. elatius*, *N. stricta* and *M. caerulea* are, respectively, 8·1, 4·9, 1·5 and 1·1, respectively.

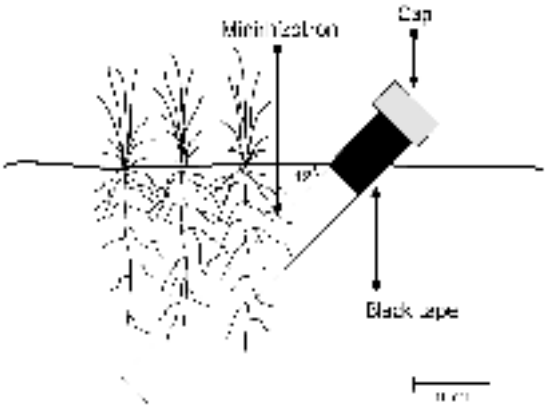

Fig. 1. Schematic presentation of the minirhizotron observation tube in the monoculture.

Sixty-four young tillers were planted in each 1 m^2 plot. In August 1993, dead plants were replaced by new tillers. In September 1993, shading gauze was erected around the plots to a height of 35 cm to keep the above-ground biomass inside the plots.

OBSERVATIONS IN MINIRHIZOTRONS

The minirhizotron technique that we used to measure root life spans and root diameters was developed by Van Noordwijk *et al*. (1985); a detailed description is given by Vos & Groenwold (1983). The technique allows the same roots to be observed repeatedly under conditions that are the best approximation of the natural state. In contrast to Vos & Groenwold (1983), the minirhizotrons we used were cylinders made of 3 mm transparent acrylate, 6 cm diameter and 60 cm long (outside measurements). In January 1995 one tube was inserted in the ground in each monoculture, at an angle of 45° to the soil surface. A cylinder-shaped piece of insulation material was placed inside each tube to prevent condensation on the inside of the minirhizotrons and to keep the inside of the tubes dark. The tubes were capped to prevent light penetrating the root environment (Fig. 1). Placement of the tubes severed a number of roots which will have stimulated root growth in the subsequent period. Plants above the roots that were followed were at a distance of at least 12 cm from the above-ground part of the tube.

Observations in the minirhizotrons started in February 1995 using an Olympus OM2 camera mounted on an endoscope. The endoscope was an extendable, rigid, 14 mm diameter instrument, with a 90° angle of reflection and a 60° angle of view. Illumination was provided by a liquid optic light guide connected to a cold 150 W light source. The light was cast on the field of view by mirrors. Camera, endoscope and light guide were mounted on a slide support, which fitted tightly into the observation tubes and could be advanced in

the tubes with increments of 5 cm. The observations continued at 2 week intervals until September 1997. On each observation date, colour images were made in each tube at fixed positions 12·4 cm below the soil surface. Each root observation position consisted of a circular area with diameter 26·3 mm. At the end of the observation period we selected roots for further analysis. The selection criteria were that roots had to be absent on the first observation date, and had to be in the centre of the slide so that they could be followed easily during their life.

DETERMINATION OF ROOT LIFE SPANS

The minirhizotron images were digitized, and contrast and brightness were optimized with image analysis software (IMAGE-PRO PLUS 2·0). Because of the large overlap in colour levels between roots and soil, the roots were traced manually. The successive minirhizotron observations on the same roots allowed us to measure root life span and changes in root diameter. One problem we had to overcome was the definition of root death. When grass roots were a few weeks old, cortical cells in the roots had begun to die, whereas the stele remained active (Deacon 1987). Moreover, these roots have been reported to absorb water and nutrients after the epidermal and cortical cells have died (Eissenstat & Yanai 1997). Even portions of roots whose entire areas of the epidermis and cortex had sloughed have been reported to provide important transport functions (Spaeth & Cortes 1995). Therefore roots were classified as dead only when we could no longer see them.

ANALYSES AND STATISTICS

We determined root life span in two ways. First, we determined root survival by monitoring the presence of the roots of the initial root cohort that appeared on the minirhizotron images between the first observation date (February 1995) and 4 July 1995. We constructed root survival curves and determined the differences in root survival between the four species, using a Gehal–Wilcoxon non-parametric test (SPSS 7·0, 1995).

Second, we monitored the presence of roots from their first appearance on the minirhizotron images until they were no longer visible. Statistical analyses were used to test whether the root life span was significantly different between the four species. We used a general linear model (SPSS 7·0, 1995). Tukey's Studentized range tests were used to test for differences among means.

Root diameter was determined by measuring it every 2 weeks at one point during the period from appearance on the minirhizotron images until the roots were no longer visible. Mean root diameter of each species was calculated for each age group. Pearson's correlation coefficients were calculated between root life span and root diameter, and between mean diameter and age (SPSS 7·0, 1995). A covariance analysis was performed to determine temporal differences in the decline of root diameter between the four species, with age as covariate (GLM procedure, SPSS 7·0, 1995). The GLM procedure was also used to determine differences between the four species in mean root diameter over the whole period.

The Pearson's correlation coefficient of the root life span versus the Clausman N index was calculated (SPSS 7·0, 1995).

Results

ROOT LIFE SPAN

Most (76–92%) of the roots visible in the minirhizotrons appeared during spring and summer; up to 8% appeared during the winter. The life span of these roots was determined in two ways. First, the overall life span of the roots that emerged during the whole year was measured. Second, the survival of one root cohort in each of the four grass species was analysed.

The overall root life span differed among the four species ($F_{3,70} = 26{\cdot}1$; $P < 0{\cdot}001$), varying from 14 weeks for *L. perenne* roots to 58 weeks for *N. stricta*. The overall life span of *L. perenne* roots was significantly shorter than that of *A. elatius*, *M. caerulea* and *N. stricta* roots ($P < 0{\cdot}001$) (Fig. 2). The life span of *N. stricta* roots was significantly longer than that of *L. perenne* ($P < 0{\cdot}001$) and *A. elatius* ($P < 0{\cdot}05$) roots. The differences in root life span between the four species were significantly negatively related with the N index of the habitats for the four species ($r^2 = 0{\cdot}95$, $P = 0{\cdot}03$; Fig. 3).

Cohort survival analyses of the first root cohorts that appeared between February and July 1995 showed the same differences. After 4 months all *L. perenne* roots in the cohort had died, but the roots of the *A. elatius*, *M. caerulea* or *N. stricta* cohorts died after 17–22 months (Fig. 4).

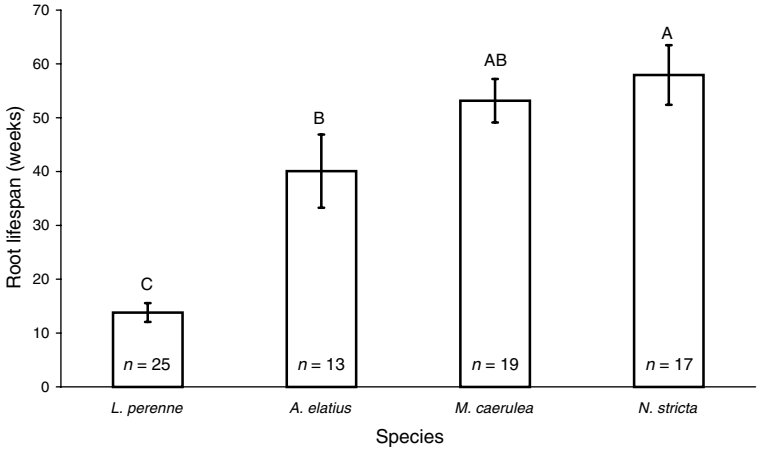

Fig. 2. Root life span (weeks) for *L. perenne*, *A. elatius*, *M. caerulea* and *N. stricta* measured in minirhizotrons in monocultures of these species. Values are means ± SE. Different upper-case letters indicate significant differences among living plants of these species ($P < 0{\cdot}05$).

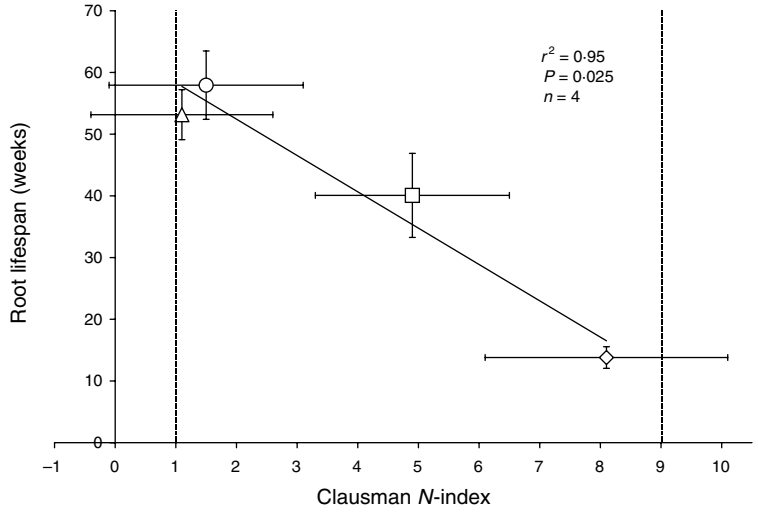

Fig. 3. Root life span (weeks) for *L. perenne* (◇), *A. elatius* (□), *M. caerulea* (△) and *N. stricta* (○) roots versus the Clausman N index of these species. Values are means ± SE. Both mean and SE of Clausman indices were borrowed from Melman *et al.* (1985). The coefficient of determination (r^2) and the significance of the correlation are given at top right.

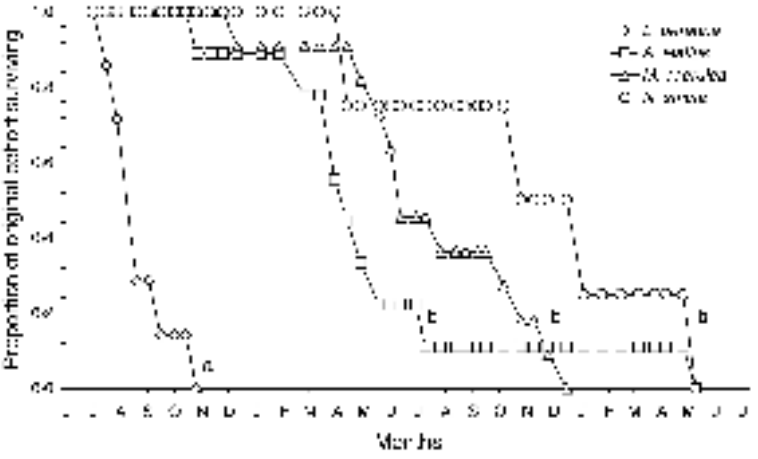

Fig. 4. Root survival curves for the root cohort produced before 4 July 1995 in monocultures of *L. perenne* (n = 7), *A. elatius* (n = 9), *M. caerulea* (n = 11) and *N. stricta* (n = 4). Lines with the same letters are not significantly different (Gehal–Wilcoxon test, $P > 0·05$).

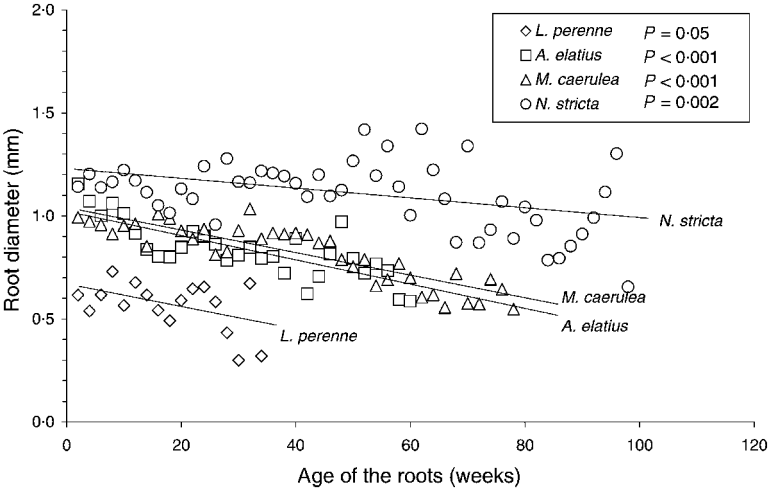

Fig. 5. Root diameter (mm) of *L. perenne*, *A. elatius*, *M. caerulea* and *N. stricta* roots measured in minirhizotrons plotted against the age of the roots. The value of significance for the correlation between root diameter and age of the roots is given at top right.

ROOT DIAMETER

Root life span increased significantly with increasing diameter of the young root (young = no more than 2 weeks old) (r^2 = 0·213, $P < 0·001$, n = 74). The root diameters of the four species differed ($F_{3,131}$ = 65·5, $P < 0·001$; Fig. 5). Overall, roots of *N. stricta* had a larger diameter than roots of *L. perenne*, *A. elatius* and *M. caerulea* ($P < 0·001$). *Lolium perenne* roots had a significantly smaller diameter than the roots of the other three species ($P < 0·001$). The root diameters of *A. elatius* and *M. caerulea* were not different (P = 0·94). In the older roots, the root diameter decreased significantly in all species (*L. perenne*: $F_{1,15}$ = 4·6, $P < 0·05$; *A. elatius*: $F_{1,28}$ = 42·9, $P < 0·001$; *M. caerulea*: $F_{1,37}$ = 121·6, $P < 0·001$; *N. stricta*: $F_{1,47}$ = 10·3, $P < 0·01$). Root diameter decreased more slowly in *N. stricta* (b = −0·007; SE = 0·002) than in *A. elatius* (b = −0·017, SE = 0·003; P = 0·02) and *M. caerulea* (b = −0·015, SE = 0·001; P = 0·004).

Discussion

We succeeded in measuring the root life span of the four grass species and its relation with the initial root diameter. The species clearly differed in their root longevity. The species from N-rich habitats, *L. perenne*, had a significantly shorter root life span than those from N-poor habitats, *M. caerulea* and *N. stricta*. Our decision to classify roots as dead only when they were no longer visible meant that we may have overestimated the absolute root life span.

Our results confirm earlier studies on root turnover (Aerts *et al.* 1989; Steltzer & Bowman 1998). However, those studies used species differing in growth form and from different families. Much of the variation between species may be associated with phylogenetic differences. It was to avoid any confounding effects of differences in growth form and phylogeny that we compared species from one family, Gramineae (see also Fransen 1999). Even for species as closely related as those that we used, there was a difference in root longevity between the slower-growing species (*N. stricta* and *M. caerulea*) and the faster-growing species *L. perenne*. Moreover, the differences in root life span between the four species were negatively related to the N index of the habitats for the four species (cf. Fig. 3).

In nutrient-poor environments the long life span of roots provides an important mechanism for plant nutrient conservation (Eissenstat & Yanai 1997). Conversely, when plants from nutrient-rich habitats have shorter root life spans, they add more C and nutrients to the soil through dead root tissues which may increase nutrient mineralization rates (Berendse *et al.* 1989; Van Vuuren *et al.* 1993).

Our study also provides evidence that, in grass species, root life span is linked to root diameter. Our finding that the species from N-rich habitats (*L. perenne*) had significantly finer roots and shorter root life spans than

the species from N-poor habitats (*N. stricta*) agrees with earlier studies showing that thin roots often die sooner than coarse roots (Eissenstat 1992). However, these studies compared thick and thin roots of the same species. A possible reason for the earlier death of thinner roots may be the costs of C allocation to the plant. If, per gram root, the C costs are the same for fine and coarse roots, then thin roots will be more efficient in nutrient uptake than coarse roots, because of the importance of root length and root surface area in nutrient and water uptake (Yanai *et al*. 1995). However, the optimal root diameter will change when C costs of fine roots exceed those of coarse roots as a result of an increasing risk of herbivory or early root death (Eissenstat & Yanai 1997).

Under nutrient-poor conditions it is especially advantageous for plant species to retain nutrients acquired at a cost of allocated plant C, by increasing the mean residence time of nutrients in the plant (Berendse & Aerts 1987). This could explain why the roots of species of nutrient-poor habitats are coarser than those of species of nutrient-rich habitats when we compare closely related species. Moreover, species of nutrient-poor habitats can reduce the risk of herbivory and parasitism by investing in a wide range of recalcitrant compounds, such as phenolics, which may increase the costs of biosynthesis. As a result these species can increase the life span of their roots (Eissenstat & Yanai 1997), while their maximum growth rate is reduced. On the other hand, under relatively nutrient-rich conditions, cheap biosynthesis may be favourable for plant species (Berendse & Aerts 1987; Chapin 1980; Poorter & Remkes 1990). Such species can respond rapidly to increased nutrient availability. Under nutrient-rich situations the presence of a large root surface area might be more important than the higher risk of root loss through herbivory.

The decrease in root diameter with age was expected to be the consequence of the early death of root cortical cells (Deacon 1987) and of the reabsorption of nutrients by the plant (Gordon & Jackson 2000). Robinson (1990) argued that root cortical death could be beneficial to plants if phosphorus is remobilized from senescing cells, but no-one has found any evidence for this. However, the rapid loss of root cortical cells is responsible for important nutrient losses from the plant.

It should be noted that the nutrient input into the soil from root turnover refers to organic N and P contained in the dead root material. To be available for plant uptake again, the litter must be decomposed and nutrients remineralized. Species differ substantially in their litter decomposability and N release from this litter (Berendse *et al*. 1989; Jensen 1996; Van Vuuren *et al*. 1993), and we have evidence for differences in root decomposability and the subsequent N mineralization between grass species (Van der Krift *et al.* 2001). The differences between species in dead root decomposability and the subsequent nutrient mineralization probably enhance the differences in nutrient input to the soil that result from root turnover.

In conclusion, our data indicate that grass species from fertile habitats lose more biomass and nutrients by root turnover, and input more C and nutrients into the soil system than species that frequently inhabit less fertile soils. The ecological consequence of such interspecific differences is that an increase in species adapted to fertile habitats will lead to an increase in soil fertility, which may lead to an accelerated expansion of the plant population involved. Such interactions may result in positive feedbacks that cause fast changes in soil fertility and plant species composition of grassland communities.

Acknowledgements

We thank F. Möller, R. Geerts, H. Klees, and J. van Walsem and the numerous summer workers for their assistance in this project, and we also appreciate the valuable comments on earlier drafts of the manuscript provided by J. Burrough, M. M. P. D. Heijmans, E. Jongejans, W. J. Roem, J. van Ruijven and G. W. W. Wamelink.

References

Aerts, R., Berendse, F., Klerk, N.M. & Bakker, C. (1989) Root production and root turnover in two dominant species of wet heathlands. *Oecologia* **81**, 374–378.

Berendse, F. (1990) Organic matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. *Journal of Ecology*. **78**, 413–427.

Berendse, F. & Aerts, R. (1987) Nitrogen-use-efficiency: a biologically meaningful definition? *Functional Ecology* **1**, 293–296.

Berendse, F., Bobbink, R. & Rouwenhorst, G. (1989) A comparative study on nutrient cycling in wet heathland ecosystems. II. Litter production and nutrient mineralization. *Oecologia* **78**, 338–348.

Bloemhof, H.S. & Berendse, F. (1995) Simulation of the decomposition and nitrogen mineralization of aboveground plant material in two unfertilized grassland ecosystems. *Plant and Soil* **177**, 157–173.

Brevedan, R.E., Busso, C.A., Montani, T. & Fernandez, O.A. (1996) Production and nitrogen cycling in an ecosystem of *Eragrostis curvula* in semiarid Argentina. II Nitrogen content and transfer. *Acta Oecologia* **17** (3), 211–223.

Chapin, F.S. III (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**, 233–260.

De Ruijter, F.J., Veen, B.W. & van Oijen, M. (1996) A comparison of soil core sampling and minirhizotron to quantify root development of field-grown potatoes. *Plant and Soil* **182**, 301–312.

Deacon, J.W. (1987) Programmed cortical senescence: a basis for understanding root infection. *Fungal Infection of Plants* (eds G.F. Pegg & P.G. Ayers), pp. 285–297. Cambridge University Press, Cambridge, UK.

Eissenstat, D.M. (1992) Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition* **15**, 763–782.

Eissenstat, D.M. & Yanai, R.D. (1997) The ecology of root life span. *Advances in Ecological Research* **27**, 1–60.

Fransen, B. (1999) Root foraging. The consequences for nutrient acquisition and competition in heterogeneous environments. PhD Thesis, Wageningen University, Wageningen.

Gordon, W.S. & Jackson, R.B. (2000) Nutrient concentration in fine roots. *Ecology* **81**, 275–280.

Grime, J.P. (1994) The role of plasticity in exploiting environmental heterogeneity. *Exploitation of Environmental Heterogeneity by Plants: Ecophysiological Processes Above- and Belowground* (eds M.M. Caldwell & R.W. Pearcy), pp. 1–20. Academic Press, New York.

Jensen, E.S. (1996) Compared cycling in a soil–plant system of pea and barley residue nitrogen. *Plant and Soil* **183**, 13–23.

Melman, T.C.P., Clausman, P.H.M.A. & de Haes, H.A.U. (1985) *Voedselrijkdom-indicatie van graslanden. Vergelijking en toetsing van drie methoden voor het bepalen van de voedselrijkdomindicatie van graslandvegetaties*. Centrum voor milieukunde, Rijksuniversiteit Leiden.

Poorter, H. & Remkes, C. (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia* **83**, 553–559.

Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1992) Leaf life-span in relation to leaf, plant and stand characteristics among diverse ecosytems. *Ecological Monographs* **62**, 365–392.

Robinson, D. (1990) Phosphorus availability and cortical root senescence in cereal roots. *Journal of Theoretical Biology* **145**, 257–265.

Schläpfer, B. & Ryser, P. (1996) Leaf and root turnover of three ecologically contrasting grass species in relation to their performance along a productivity gradient. *Oikos* **75**, 398–406.

Spaeth, S.C. & Cortes, P.H. (1995) Root cortex death and subsequent initiation and growth of lateral roots from bare steles of chickpeas. *Canadian Journal of Botany* **73**, 253–261.

Steltzer, H. & Bowman, W.D. (1998) Differential influence of plant species on soil nitrogen transformations within moist meadow alpine tundra. *Ecosystems* **1**, 464–474.

Troughton, A. (1981) Length of life of grass roots. *Grass and Forage Science* **36**, 117–120.

Van der Krift, A.J., Gioacchini, P., Kuikman, P.J. & Berendse, F. (2001) Effects of high and low fertility plant-species on dead root decomposition and nitrogen mineralization. *Soil Biology and Biochemistry* **33**, 2115–2124.

Van Noordwijk, M. & de Jager, A.& Floris, J. (1985) A new dimension to observations in minirhizotrons: a stereoscopic view on root photographs. *Plant and Soil* **86**, 447–453.

Van Vuuren, M.M.I., Aerts, R., Berendse, F. & de Visser, W. (1992) Nitrogen mineralisation in heathland ecosystems dominated by different plant species. *Biogeochemistry* **16**, 151–166.

Van Vuuren, M.M.I., Berendse, F. & de Visser, W. (1993) Species and site differences in the decomposition of litters and roots from wet heathlands. *Canadian Journal of Botany* **71**, 167–173.

Vos, J. & Groenwold, J. (1983) Estimation of root densities by observation tubes and endoscope. *Plant and Soil* **74**, 295–300.

Wedin, D.A. & Tilman, D. (1990) Species effects on nitrogen cycling: a test with perennial grasses. *Oecologia* **84**, 433–441.

Yanai, R.D., Fahey, T.J. & Miller, S.L. (1995) Efficiency of nutrient acquisition by fine roots and mycorrhizae. *Resource Physiology of Conifers* (eds W.K. Smith & T.M. Hinckley), pp. 75–103. Academic Press, New York.

Received 4 April 2001; revised 27 August 2001; accepted 5 September 2001