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BIOMASS ALLOCATION AND NITROGEN DISTRIBUTION IN RYEGRASS UNDER WATER AND NITROGEN SUPPLIES

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□ *Ryegrass* (Lolium perenne *L.) in grassland is known to sustain with water and nitrogen (N). This study investigates biomass and N partitioning in plant organs (roots, main and the youngest tillers) under water-nitrogen interactions. Nitrogen was applied at the rates of 50 and 100 mg N kg^{-1} as N_1 (low N) and N_2 (high N) treatments, respectively, with uniform irrigation until 440 growing degree-days (GDD). Thereafter, the water supply was restricted to 50 mL on a weekly basis (W_1) against 50 mL on a daily basis (W_2) and concurrently, N enriched with 1 atom% ^{15}N isotopes. Cumulative tillers' biomass increased linearly from 1st to 8th order, but thereafter reached a plateau with further increases in number of negligible weights. Initially tiller mass and number per plan did not differ ($P < 0.05$) with water and/or N applications but changed at 788 GDD with clear differences at 911 GDD with the highest under N_2W_2 and lowest under N_1W_1. Nitrogen concentration sharply decreased from 530 to 700 GDD and then levelled off with age. The decline was more pronounced in tillers than roots. The high N treatment showed elevated N-concentration under both water treatments. Watering on a daily basis promoted vegetative growth. High water and N levels significantly ($P < 0.05$) influenced concentration of N absorbed during ^{15}N labeling (N_L) in all organs with relatively pronounced N_L under N_2. The additive positive effect of W_2 and N_2 was obvious on N_L as compared to N_T, which showed that plants discriminate N-uptake on mass basis. Nitrogen (mobile) was higher in young and ^{15}N (heavier) was low in young tillers and vice versa. Accumulation of N absorbed during ^{15}N labeling ($^{15}N_A$) was significant knowing that water is a strong determining factor of N concentration in ryegrass organs.*

Keywords: dry matter distribution, total nitrogen, water stress, ^{15}N distribution, ^{15}N accumulation, ryegrass tillers

INTRODUCTION

Water-nitrogen (N) relationship is a useful tool in the management of water and N application for optimizing crop productivity (Kibe et al., 2006). Water deficiency is an important factor, limiting production in many areas

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of the world, but its effect is more severe in arid and semiarid regions than humid regions (Beinhauer and Guenther, 1990). Nitrogen is a complex part of the soil system and its availability to plants is affected by soil type, tillage, N source, crop rotation, precipitation, and method and type of fertilizer applications (Hatfield et al., 2001). Water shortage in crops can reduce dry matter and tiller number in cereals such as wheat (*Triticum aestivum* L.) (Musick and Duseck, 1980; Sommer et al., 1990), and ryegrass (*Lolium perenne* L.) (Luxmoore and Millington, 1971; Colman and Lazemby, 1975). However, the interactive effect of water and N has not been investigated for tiller per plant. Metabolic processes leading to increases in vegetative and reproductive growth and yield depends on the adequately supply of N (Cechin and Fumis, 2004). Both field and laboratory investigations show increasing N applications to promote growth and photosynthesis. Reduction in plant growth due to low N is associated with reduced leaf production, leaf area and tillers numbers (Hatfield et al., 2001). Under adverse climatic conditions, grasses compensate for reduced photosynthetic rates and N accumulation by utilizing previously reserved compounds for growing tissues such as leaves and tillers.

Partitioning of N to shoots and roots is an important mechanism for plants adapting to a particular environment. Water and N availabilities affect root:shoot ratio. When the N supply increases, the root:shoot ratio in grasses decreases because the leaf number and area per tiller increase rendering the plants vulnerable to water stress (Akmal, 1997). Limited water supply reduces shoot growth more than root because of the solute accumulation at root tips maintaining high turgor pressure for plants survival (Sharp and Davies, 1979; Akmal and Hirasawa, 2004). Yield of forage crops is measured primarily in terms of dry matter production (Nelson and MacAdam, 1989). The herbage protein and digestibility are influenced by water and N availability during growth (Abdul-Kadir and Paulsen, 1982). The study of stable isotopes has made the close monitoring of carbon (C) and N partitioning in various plant organs under different environmental condition possible (Schnyedr and de Visser, 1999; Lattanzi et al., 2005; Collins, 2007). Through utilization of stable isotopes of ^{15}N it is easy to monitor N distribution in plant organ at any growth stage. It is of interest to know how dry matter and N are distributed within ryegrass during early vegetative growth under low and high N and water applications. Therefore, the focus of this study was to investigate the interaction between water and N on uptake and accumulation of N in growing as well as mature tillers using stable isotopes of ^{15}N.

MATERIALS AND METHODS

Experimental Design and Sowing of Ryegrass

The experiment was conducted at the Institute of Agronomy and Crop Science, University of Bonn, Germany. Ryegrass (cv. 'Liprenta') was planted

in pots (25 cm height and 10 cm diameter) on 14 August 1994. All pots were filled with 2.5 kg substrate of thoroughly mixed soil and sand (2:1 v/v). The soil was clay loam, collected from an experimental plot of the University Research Station 'Dikopshof" having very low total N content (0.072%). The quartz sand had a particle size of 0.4–0.8 mm and was purposely mixed to allow examination of root material. To get a uniform canopy structure resembling a field, 144 pots (i.e., two water × two N × four replications × with maximum of nine samplings for the study duration) were arranged in randomized complete design on a trolley in the open air. The trolleys were covered with polyethylene sheets to avoid interference through rainfall without preventing solar radiation. Two extra rows of pots were also provided on either side to avoid border effect on the treatments canopy. Plants were sheltered with a transparent polyethylene sheets preventing rain interference. Each pot was sown initially with 20 seeds and thinned to 10 seedlings after emergence. Four pots per treatment, representing four replications of a treatment, were harvested at random for a specified data collection. Growing degree-days (GDD) were calculated following Russels et al. (1984) except for using 4°C as base temperature while ryegrass has shown growth in cold winter nights (Akmal and Janssens, 2004). Phosphorus (P; 1.5 g m^{-2}) and potassium (K; 4.8 g m^{-2}) were applied to all pots at the time of sowing in solution form using monopotassium phosphate (KH_2PO_4) and potassium chloride (KCl) (Alvenaes and Marstorp, 1993).

Nitrogen and Water Treatments

Two N treatments, 50 and 100 mg pot^{-1} hereafter called N_1 and N_2, respectively, prepared from calcium nitrate [$Ca(NO_3)_2.4H_2O$] in de-mineralized water, were applied 10 times in 20 mL pot^{-1}. The first N solution was applied right after emergence, i.e., 10 days after sowing (DAS) followed by subsequent applications on 17, 27, 36, 45, 53, 62, 70, 79, and 87 DAS for a total growing season of 94 days. The first N applications (10, 17, and 27 DAS) were comprised of normal N, while the remaining N applications (starting 36 DAS) were enriched with 1% ^{15}N obtained from $Ca(NO_3)_2.4H_2O$ (^{15}N, atom 10%). The ^{15}N applications were made on the days when subsequent samples were harvested and coincided with the amount of irrigation. An automatic water supply system irrigated each pot. Each pot was connected through a capillary tube to the main irrigation supply using and automatic water supply system. The main supply was further connected to pump submerged in reservoir of the de-mineralized water replenished on weekly basis. The pump was programmed to discharge 50 ml water pot^{-1} uniformly as per crop demand till 36 DAS (440 GDD), after which water stress was imposed supplying 50 mL water pot^{-1} $week^{-1}$ (W_1) and 50 mL water pot^{-1} day^{-1} (W_2).

Sampling Procedure

Plant dry matter was collected at weekly intervals starting 45 DAS, i.e., a week after the first ^{15}N application and imposition of W_1 and W_2 treatments. On each sampling date, all plant material including roots from each pot were thoroughly washed and superficially dried with tissue paper for an hour to extract extra water adhered during washing. Five out of ten plants having uniform morphological appearance were dissected for further observations. Each plant was separated into roots and tillers. The main tiller was identified on the basis of highest mass and leaf number. The youngest tillers were the one with lowest masses and still remaining within the leaf axils of the mother tiller (Briske, 1991). All tillers and roots of a plant were dried at 60°C for about 40 h and weighed for dry mass determination. Total dry matter of a ryegrass plant was estimated as cumulative masses of all tillers including root weight.

Of the five dissected plants pot^{-1}, three representative plants were selected for total N concentration and ^{15}N determination. Roots, main tiller, second last tiller and the last tiller of a plant were ground using a small ball grinder (Retsch GmbH, Hann, Germany). The dried ground samples were weighed in tin cups (Type 76 9813 26, Lüdi, Flawil, Switzerland) and analyzed for total N and ^{15}N using an automatic elemental gas chromatograph interfaced to a continuous flow isotope-ratio mass spectrophotometer (Roboprep TCD-tracermass; Europa Scientific, Crew, UK). The concentration of N absorbed during ^{15}N labeling (N_L) was determined from procedure adopted by Schaeufele (1996) using the following equation:

$$N_L = N_T \times \left[(^{15}N_{PS} - ^{15}N_{NAT}) \div (^{15}N_{PAP} - ^{15}N_{NAT}) \right] \quad (1)$$

where N_T is the tissue N concentration [% in sample], $^{15}N_{PS}$ is the atom percentage ^{15}N of N in the samples, $^{15}N_{NAT}$ is the atom percentage of ^{15}N naturally existing in the sample equal to 0.366 (Deleens et al., 1994) and $^{15}N_{PAP}$ is the atom percentage ^{15}N of N fertilizer in the (labeled) nutrient solution.

The accumulation of N absorbed during ^{15}N labeling (N_A mg g^{-1}) was estimated for the maximum dry matter stage by multiplying corresponding values of the concentration of N during ^{15}N labeling of the plant organs.

Calculations and Analysis

A modified Richards' equation (Richards, 1959) was fitted considering tiller order (i.e., age on the basis of tiller mass and leaf number per plant) as independent variable and their dry mass as dependent variable. The

TABLE 1 Richards' function constants obtained from equation fit for plant tillers' masses and ranking position from main tiller in ryegrass (*Lolium perenne* L.) supplied with the different N (low and high) and water levels (insufficient and sufficient) during vegetative growth ($r^2 = 0.99$)

Treatment		Variable			
Water	Nitrogen	a	b	c	k
W1	N_1	0.52	1199	1.10	5.12
	N_2	0.57	1198	1.13	5.11
W_2	N_1	0.61	1196	1.09	5.11
	N_2	0.70	1194	1.05	5.07

following equation was used for a smooth dry matter development curve:

$$y = a/(1 + b * \exp(-c * x)^{(1/k)} \quad (2)$$

where y is the tiller mass plant^{-1} at nth tiller order, a is maximum tiller cumulative dry mass possible under prevailing environmental conditions (upper asymptote of the curve), c is the time at which maximum growth occurs, and b is the weighted mean relative growth rate. Values obtained for different constants of the Richard's functions are shown in Table 1.

Analysis of variance was carried out using GLM procedure of the SAS (SAS Institute, 1996). Data analyses were performed collectively for all sampling exercised during the study period of the experiment. Treatments and/or their interactions were tested using Tukey's Studentized Range (HSD) at 5% significant level. Nonlinear regression of the different curves were made using Sigmaplot (version 8.0; Systat Software, Chicago, IL, USA).

RESULTS AND DISCUSSION

The dry matter and its partitioning in different tillers of the ryegrass plant supplied with two levels each of N and water application, during the vegetative growth is shown in Figure 1. The Figure represents data of cumulative mass of tillers collected after about two weeks at given sampling dates ranging from 530 to 911 GDD in relation to tiller order. The tiller orders were characterized on the basis of tiller mass and leaf number ranked from 1 to nth plant^{-1}, i.e., tiller 1 represents the main tiller and increasing number up to nth mass subsequent daughter tillers. Therefore, the values are the cumulative mass of main tillers plus the subsequent daughter(s) tillers. Cumulative mass of the tillers increased linearly with increasing tiller order up to 7th and 8th and beyond that mass of the ryegrass plant did not change with increasing order showing positive correlation coefficient ($r^2 = 99$). Figure 1 indicates that beyond a certain point the mass of the ryegrass plant

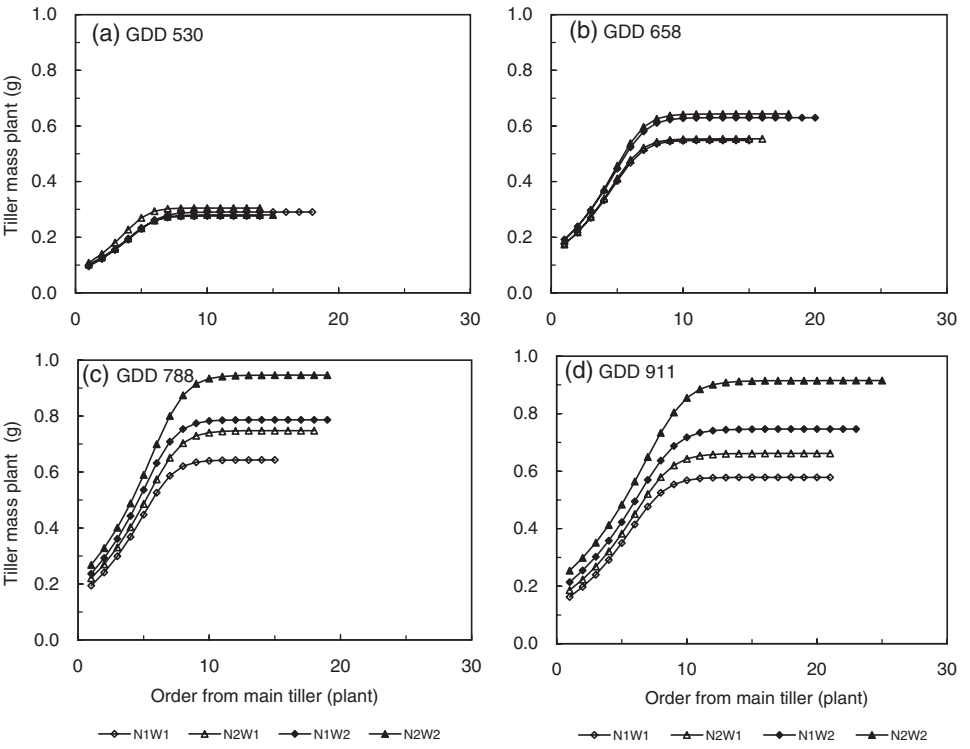

FIGURE 1 Relationship between cumulative mass of the plant tillers in relation to order of ryegrass (*Lolium perenne* L.) supplied with low (N_1) and high (N_2) nitrogen and low (W_1) and high (W_2) water during vegetative growth. Different parts of the figure indicate sampling made on 45 (530 GDD) 62 (658 GDD) 79 (788 GDD) and 94 (911 GDD) days after sowing. Regression lines were made using the Richards' function (n = 20).

did not change because order of tiller increased due to the smallest invisible tiller number with little mass (Langer, 1979; Briske, 1991). The linear increase is primarily associated with the main tiller order.

On the first sampling (530 GDD), both tiller mass and number did not differ significantly ($P < 0.05$) with water and N treatments application and for their interactive effects (Figure 1a). At 658 GDD, a significant effect on tiller mass was observed with high (W_2) water application rates (Figure 1b). The high N application (N_2) effect became significant at 788 GDD on tillers' mass at both levels of water (Figure 1c) and became more distinct with advancement in growth at GDD 911 under N_2W_2 as compared to N_1W_1 (Figure 1d). At given level of N, the increasing water supply from W_1 to W_2 produced twice as much dry mass as produced by increasing N_1 to N_2 at given level of water at 911 GDD (Figure 1d). The difference in dry matter of the ryegrass plants under N and water application rates was associated with changes in main tillers, followed by a more or less moderate change in the adjacent 7 to 10 daughter tillers of the plants in ranking order by weight

(Figure 1d). It appears that at the time of the first sampling (DGG 530) the effect of N and water treatments are not distinctly visible (Figure 1a) due to low requirements of the plant for water and N because of smaller plant size. As the plant growth progressed, the effect of water and N and their interaction showed their significant ($P < 0.05$) effect on tiller mass as their demand was adequately supplied (Figures 1b–1d). This explanation is consistent with commonly reported function of N (Frota and Tucker, 1978; Hatfield et al., 2001; Lowlor, 2002; Guenni et al., 2002; Ma et al., 2006) and water (White et al., 1993; Carrow, 1996; Huang and Fry, 1998; Apr et al., 1998).

Total nitrogen concentration (N_T) in roots and tillers sharply decreased with increasing GDD from 530°C to 700°C and then leveled off with growth and time (Figures 2a to 2c) except the last tillers which slightly increased at GDD 911 (Figure 2d). This initial decline was more pronounced in tillers as compared to roots, which maintained two to three times lower N_T than

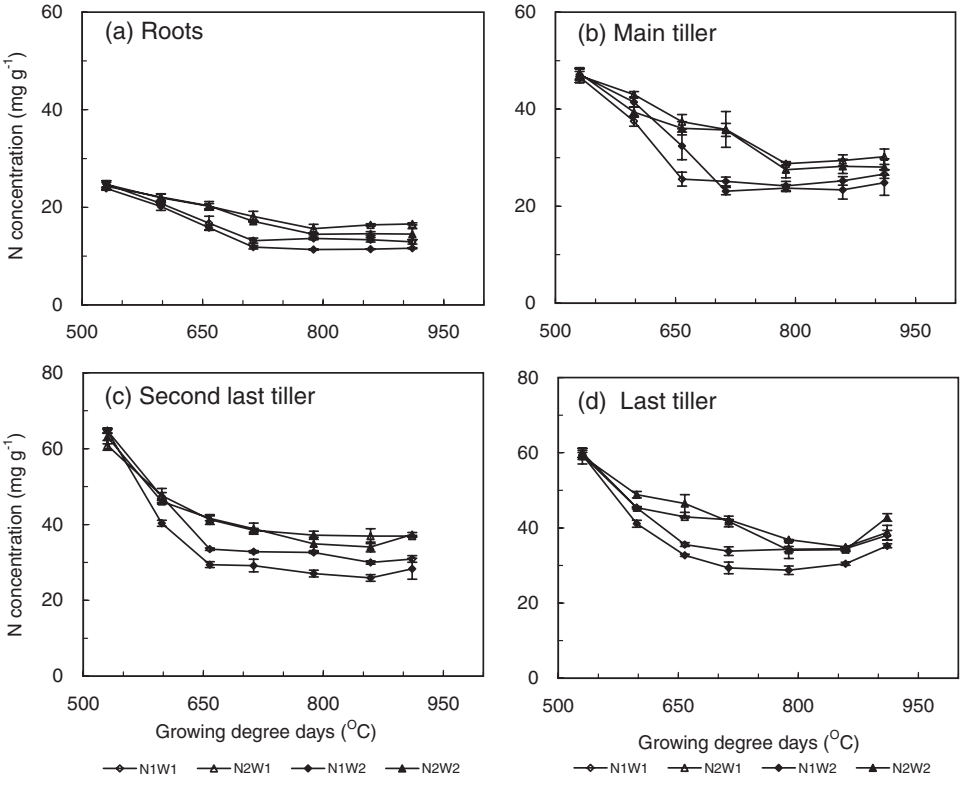

FIGURE 2 Tissue nitrogen concentration (mg N g^{-1} DM) in plant organs during vegetative growth supplied with low (N_1) and high (N_2) nitrogen and low (W_1) and high (W_2) water during vegetative growth. Different sections of the figure present different organs of the ryegrass (*Lolium perenne* L.) plant: (a) roots, (b) main tiller, (c) second last tiller, and (d) last tiller. Vertical bars are standard deviation, where no bar is visible, the SD is smaller than symbols.

tillers. The addition of high N (N_2) produced higher N_T than N_1 at both high (W_2) and low (W_1) water treatments while the concentration of N_T was lower at W_2 as compared to W_1 at N_1 and N_2 during the later stage of growth. As evident from Figure 1a, the main tiller showed sharp increases initially and then remained unchanged while the N_T concentrations followed the pattern indicating the dilution effect due to plant size. As the plant size remained constant the N_T also did not change with increasing GDD (Figure 2a–d). When the growth rate of a plant exceeds the rate of nutrient uptake the concentration of the nutrient decreases, this is popularly known as the dilution effect (Lemaire and Gastal, 1997; Gastal and Lemaire, 2002). This explanation is supported by the effect of high water level and N_T at given level of N. The low N_T level observed at N_1W_2 as compared to high N_T observed at N_1W_1 is associated with higher plant size (mass) due to high water application and vice versa. In other words, the application of water at W_2 on daily basis promoted rapid plant growth as compared to W_1 where water was added on weekly basis. The initial vegetative growth of ryegrass plants strongly depend on water supply (Hebblethwaite, 1977; Akmal, 1997) and increases curvilinear with N application depending on plant size, age and mode of nitrogen application, crop rotation and precipitation (Oberle and Keeney, 1990; Reeves et al., 1993; Hatfield et al., 2001).

Irrespective of water and N supplies, new N concentration (N_N) increased with increasing GDD in all the plants organs (Figure 3). N_N, derived using Equation (1), was lower by a factor of two in roots compared to the tillers and was highest in youngest tillers (Figures 3c–3d) than the main tillers (Figure 3b) of ryegrass plants. Water and N levels significantly ($P < 0.05$) influenced N_N in all plant organs. When averaged across water treatments, the concentration of N_N increased by 80.5% in roots, 76.2% in main tiller, 66.7% in second last tiller and 62.5% in the last tiller at GDD 911 with N_2 as compared to N_1. The effect of high water application on N_N was more pronounced at N_2 as compared to N_1 in all organs. It was estimated that plants supplied with W_2 yielded 6.9%, 13.2%, 9.3% and 20.0% greater N_N in roots, main, second last and the last tillers, respectively, as compared to W_1 treatment, when averaged across N treatments. The additive positive effect of high water and high nitrogen treatments was more obvious on N_N as compared to N_T. It is important to note that N_T sharply decreased at 650 GDD and than maintained little change with growth while N_N tended to increase with growth period. Secondly plant tillers maintained several fold higher concentration of N_T (20 to 06 mg g^{-1}) as compared to N_N (2 to 25 mg g^{-1}). This observation suggests that plants has discriminated the uptake of nitrogen on mass basis (Stevens et al., 2005; Collins et al., 2007). The N_T representing ^{14}N being mobile was higher in younger tillers than the older mother tillers while ^{15}N being heavier was lower in younger tillers and increased in older tillers.

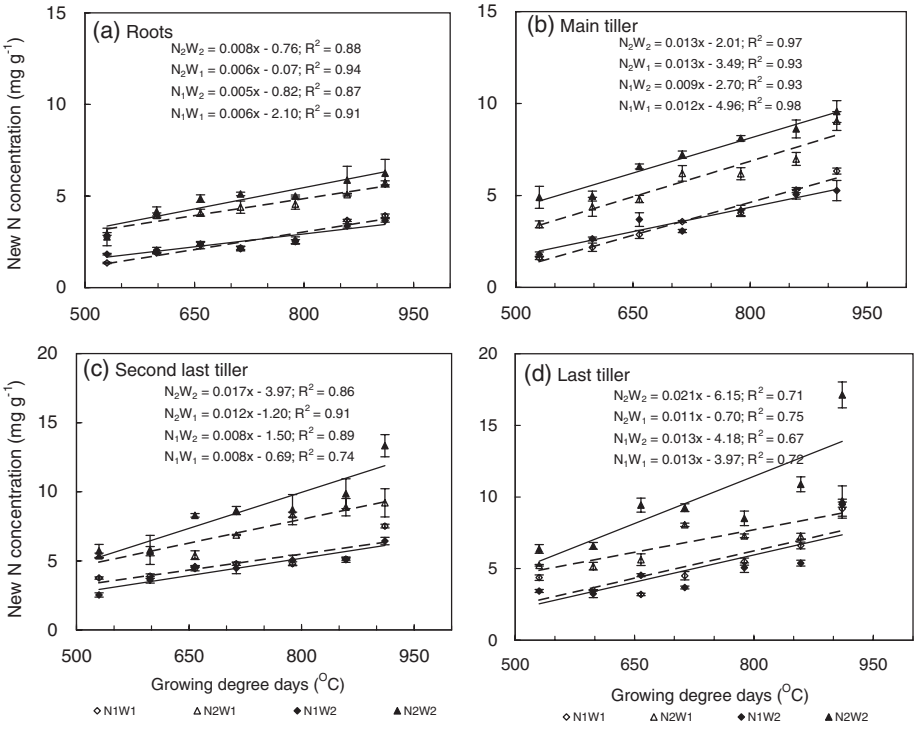

FIGURE 3 New nitrogen concentration (^{15}N labeled) in plant organs of ryegrass (*Lolium perenne* L.) during vegetative growth supplied with low (N_1) and high (N_2) nitrogen along with insufficient (W_1) and sufficient (W_2) water supply. Different sections of figure present results for different organs: (a) roots, (b) M_T, (c) L_{T-1} and (d) L_T. Vertical bars are standard deviation, where no bar visible, the SD is smaller than the symbol.

New N accumulation (N_A) was calculated for peak biomass production stage (788 GDD) of the plant growth. A significant effect on N_A was found in roots and main tillers under different water and N supply along with their interactions (Table 2). The N_A in young developing tillers of the grass was influenced ($P < 0.05$) by N supply only. Increasing N supply from N_1 to

TABLE 2 New nitrogen accumulation in ryegrass (*Lolium perenne* L.) supplied with low and high nitrogen along with insufficient and sufficient water during vegetative growth at the maximum DM production (Growing Degree Days = GDD 788)

Treatments		Roots	Main tiller	Second last	Last tillers
Water	Nitrogen	[mg ^{15}N Organ^{-1}]			
W_1 Insufficient	N_1	21.9 c	20.3 c	2.30 a	1.70 a
	N_2	35.1 b	29.0 bc	4.80 a	2.60 a
W_2 Sufficient	N_1	37.8 b	30.9 b	3.80 a	1.60 a
	N2	59.7 a	53.9 a	5.50 a	2.80 a

Means followed by common letters are not statistically different ($P < 0.05$).

N_2 enhanced N_A by 59% in roots, 62% in the main tillers, 67% and 64% in the second last and last tillers when averaged across water treatments. The higher water supply increased N_A by 70% and 72% in roots, 52% and 86% in main tiller, 65% and 14% in the second last tiller and about −2% and 7% in the last youngest tiller of ryegrass plant when they were provided with low and high N, respectively, which again supported the effect of plant size and the effect of N supply on the uptake of N_T.

The study suggests that changes in dry matter of plants with low and high water or N is primarily associated with significant reduction in mass of main and subsequent daughter tillers of almost the same size. However, total tiller number plant^{-1} does not play significant role in plant mass contribution under either low water or N supplies. Between water and N treatments, water is a strong determining factor of N concentration in the roots and shoots production. Water application rate during the vegetative growth appears to be critical determining factor of N_N accumulation. However, the experiment did not consider N partitioning among tillers because data do not allow any conclusions that how much ^{15}N labeled has been turned over in roots and other older tillers as well as partitioned to the growing tissues. According to ^{15}N labeling experiments with *Lolium perenne* identifying N partitioning during re-growth after defoliation, we considered a possible translocation from older main tillers to the youngest tillers throughout the growth period. Moreover, the ^{15}N in the youngest tillers did not come from the same source, it was translocated from the older to the younger tillers and also absorbed from the roots directly.

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