Assessment of the nitrogen status of grassland

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Abstract

Two types of diagnostics are used for N management in grasslands: diagnostics based on N concentration of shoots and diagnostics based on soil mineral N. The Nitrogen Nutrition Index (NNI) is an example of the first type. However, its evaluation requires the determination of shoot dry weight per unit area and, thus, constitutes a practical limit to its utilization in the context of farm studies. In order to simplify its evaluation, a method based on the N concentration of the upper sward layer (N_{up}) has been proposed. The objectives of this study were to test the relationship between NNI and N_{up} in the context of permanent grassland and to examine the relationship between N_{up} and soil mineral status. The study was conducted as two experiments, one on small cut-plots receiving contrasting rates of mineral N fertilization, and a second on plots of an existing field-scale lysimeter experiment. In each plot and at several dates, shoot biomass within quadrats was measured, N concentration was determined on the upper leaves and on the entire shoots, and mineral nitrogen of the soil below the vegetation sampled was determined. N concentration of the upper lamina layer of the canopy was linearly related to the NNI determined on the entire shoots. Therefore, determining N concentration in leaves at the top of canopy appears to be an alternative means to evaluate NNI without having to measure shoot biomass. The absence of an overall significant correlation between soil mineral N content and sward N index, observed over the two studies, indicates that each of these two indicators has to be considered specifically in relation to the objective of the diagnostic procedure. As sward N index may vary independently of soil mineral N content, the sward N indicator does not appear to be a suitable indicator for diagnosis of environmental risks related to nitrate leaching. However, soil mineral N content does not allow the prediction of sward N status and thus is not a suitable indicator of sward growth rate. Although soil mineral N content is an important environmental indicator for nitrate-leaching risks during potential drainage periods, it has a limited diagnosis value with respect to the herbage production function of grasslands.

Keywords: nitrogen status, grasslands, nitrogen index, plant nutrition, soil mineral N

Introduction

The nitrogen status of grassland is not only a measure of its productivity but also of its potential for polluting the environment through N losses to water and to the atmosphere. Increasing N input to grasslands increases N losses disproportionately to sward production (Scholefield *et al.*, 1991, 1993; Vertes *et al.*, 1997). In order to minimize N losses, N supply to the sward has to remain below its N uptake potential. Therefore, there is a need to develop simple and reliable field usable tools to identify the critical levels of N supply at different sites and under various management practices.

The search for and evaluation of diagnostics tools for N management in agriculture has been the subject of much attention throughout Europe (Jarvis, 1997). Current diagnostic tools are essentially based either on measuring plants or soil mineral N status (Scholefield and Titchen, 1995). Diagnostics based on plants rely either on the determination of total N concentration in plant tissues (Lemaire and Gastal, 1997), or on the determination of nitrate concentration in the sap (Lyons *et al.*, 1992) or in the stem (Justes *et al.*, 1997). Nitrate-sap tests have generally been developed for vegetable crops (Olsen and Lyons, 1994; Waterer, 1997; Belec *et al.*, 2001). They are often found to be cultivar- and growth stage-specific (Waterer, 1997; Williams and

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Maier, 2002), and are mostly relevant under situations of intensive N supply. Grasslands are generally exposed to less intensive N supply situations. Furthermore, ammonium represents a substantial proportion of mineral nitrogen in many grassland soils, and nitrate is not always the major form of mineral N taken up by the sward. Therefore, nitrate tests are not as suitable for grasslands as they are for other crops, and plant tests in use mostly rely on the analysis of plant N concentration.

A Nitrogen Nutrition Index (NNI) has been derived from plant N concentration. This index is based on the concept of critical nitrogen concentration (N_{crit}), defined as the minimum shoot N concentration necessary to obtain maximum growth rate. Values of N_{crit} are high at the start of the growing period and decline during growth, in relation to dry matter accumulation (DM, t DM ha^{-1}) according to the following equation (Lemaire and Salette, 1984):

$$N_{crit}(\%) = 4{\cdot}8(DM)^{-0{\cdot}32}.$$

This equation has been validated on several grass monocultures including tall fescue and cocksfoot (Lemaire and Denoix, 1987), on a number of multi-specific permanent swards (Duru and Thelier-Huche, 1997) and on several arable crops (for a review see Gastal and Lemaire, 2002). The NNI is calculated as the ratio of the actual N concentration of the sward to the N concentration it would have to be, at a similar biomass, to sustain a non-limiting growth and biomass accumulation (Lemaire and Gastal, 1997). For practical N management on grasslands, NNI thresholds have been defined: NNI values above 1 indicate that N nutrition is 'excessive', NNI values between 1 and 0·8 indicate that N nutrition is 'satisfactory', and values below 0·8 indicate that N nutrition is significantly limiting sward growth (Duru *et al.*, 1997).

However, the evaluation of NNI requires the determination of shoot biomass per unit area, in addition to the determination of the shoot N concentration. The determination of shoot biomass per unit area is time-consuming and constitutes a practical limit to using NNI on farms. In order to simplify the evaluation of NNI, a method based on the determination of the N concentration in the upper leaves has recently been proposed (Gastal *et al.*, 2001). Considering that the decline in N_{crit} with biomass accumulation during growth is primarily due to a decrease in the leaf:stem ratio, and secondarily to acclimation of leaf N to light within the canopy (Lemaire and Gastal, 1997), this new method is based on the principle that the N concentration in leaves at the top of the canopy remains constant during growth. Actually, N concentration of the upper layer of the canopy (N_{up}) was found to be linearly related to the NNI determined in the entire shoots on pure perennial ryegrass and tall fescue swards (Gastal *et al.*, 2001).

The validity of N_{up} as an easier and reliable diagnostic of the N status of grassland appears promising but there are several questions about its domain of validity that require investigation. One is whether N_{up} and NNI are well correlated for permanent pastures containing several species. A second concerns the utility of plant N as opposed to soil N diagnostics for the purpose of guiding N management on the farm.

The objectives of this study were first to test the relationship between NNI and N_{up} in the context of permanent grassland with several species, under a range of N fertilizer application rates and under several sward management situations. Secondly, it was to examine the relationship between N_{up} and soil mineral N in relation to the potential use of N_{up} for both retrospective and predictive assessment of sward N status.

Materials and methods

Experimental site, treatments and plant, and soil, sampling

The two experiments were conducted in 2000 at a field site 2 km from the Institute of Grassland and Environmental Research, North Wyke, Devon, UK (50°45′N, 3°55′W). They were undertaken on adjacent plots of the site, one on small cut-plots newly receiving contrasted rates of mineral N fertilization, and a second on plots of a field-scale lysimeter experiment. For both experiments, the soil was classified as a poorly drained clay loam soil of the Hallsworth series (FAO stagno-districgleysol) with the Ap horizon to the approximate depth of sampling containing 38% clay, 50% silt and 12% sand (Scholefield and Titchen, 1995).

Experiment 1

For Experiment 1, small plots were set up at the beginning of April 2000 on a sward which was at least 50 years old, with no history of disturbance by arable cropping, and dominated by *Agrostis* spp., *Holcus lanatus*, and perennial ryegrass. This sward had received no N fertilizer for nearly 15 years, but it had been grazed each year from the end of June, at a low stocking rate. Three levels of N (0, 60 and 120 kg N ha^{-1}, subsequently referred to as 0, 60 and 120 N, respectively) were applied on 10 m^2 plots in four replications. The highest N level (120 N) corresponds to the fertilizer recommendation for moderate N-supply soils. In addition, 2 t ha^{-1} lime, 60 kg ha^{-1} P and 100 kg ha^{-1} K were applied to all treatments to avoid limiting mineral factors others than N.

In each plot, biomass was evaluated at three different dates during the spring growth (11, 24 and 31 May) and at three other dates during the summer (13, 26 July and 8 August), by cutting with pruning shears to a height of 4–5 cm within a quadrat of 0·15 m^2 (0·3 m × 0·5 m). On the four outer boundaries of each quadrat, handfuls of herbage were selected and cut with scissors at 10 cm from the tip of the longest leaf. This sampling procedure combined the practicality of a grab sample and minimization of the light gradient for the leaf sample. When any stems, flowers, seed heads or senescent leaves were present in the sample, particularly by the end of the spring, they were discarded from the sample. At each date, two 25 mm diameter soil cores to 30 cm depth were taken within the quadrat immediately after the biomass sampling. The 30 cm sampling depth was chosen in the light of previous measurements which showed that typically more than 0·90 of the mineral N present in the soil profile to 1·0 m is present in this layer (Titchen and Scholefield, 1992). At the three summer samplings, in order to analyse separately the various grass species, all the herbage was cut to ground level, the species were sorted in the laboratory, and handful of each species were cut at 10 cm from the tip of the longest leaf.

Experiment 2

In Experiment 2, the plots used were established in 1982. At the time of this study, their management consisted of 1 ha paddocks divided into 10 sectors 0.1 ha each, which were either grazed, cut, or grazed and cut. The paddocks were permanent swards with the same species as in the first study. The nitrogen treatments were a conventional fertilizer programme (CONV) receiving 280 kg N ha^{-1} year^{-1}, a 'best nitrogen management practice' (BNMP), and a 0 N treatment (Table 1). The aim of the BNMP treatment was to match fertilizer inputs with predicted supplies from soil N to achieve optimum herbage production with a 0·20 reduction in fertilizer N input. In addition, farmyard manure (FYM) was applied conventionally in the autumn to the cut areas, but the BNMP treatment received composted FYM after the first and second silage cuts. The CONV or BNMP nitrogen treatments were applied to drained and undrained plots. Overall, measurements were taken in 'cut' and in 'cut and grazed' sectors of four paddocks receiving the following managements: CONV/undrained, CONV/drained, BNMP/undrained and 0 N. Silage cuts were taken on 11 June and 12 August 2000. All the plots received additional P and K fertilization. During the period April–August 2000, the plots were sampled on six occasions (11 April, 23 May, 26 June, 4 July, 27 July and 8 August). On each date, five samples per plot were taken, as described for Experiment 1, in order to determine N concentration of the upper leaves of the sward and soil mineral N content. Whole herbage samples were taken in quadrats on one occasion (23 May).

Table 1 Amount of nitrogen fertilizer applied (kg ha^{-1}) in 'best management practice' (BNMP) and conventional fertilizer programmes in undrained and drained soils in Experiment 2.

	Management treatment		
	BNMP undrained	Conventional undrained	Conventional drained
11 March	0	103	103
21 March	55	0	0
2 May	90	0	0
9 May	0	68	68
Total before silage	145	171	171
16 June	47	97	97
7 July	0	0	0
16 August	0	0	0
Total of year	192	268	268

Analytical methods

Fresh herbage samples were oven-dried at 85°C, weighed and ground. Total N concentration was determined in these dried herbage samples according to the Dumas method, with a C-N elemental analyser (model NA 2000; Carlo Erba, Milan, Italy). Soil samples were dried at 80°C and weighed. Subsamples (100 g) were taken and extracted with 200 ml of 2·0 M KCl with a shaker for 1 h (Whitehead *et al.*, 1981). The extracts were filtered and analysed with an auto-analyser (Skalar, Breda, The Netherlands) for nitrate and ammonium.

Results and discussion

Nitrogen Nutrition Index of the swards

Experiment 1

In Experiment 1, shoot N concentration declined with biomass accumulation during growth for each nitrogen fertilizer treatment (Figure 1, spring period), and increased with increasing nitrogen supply. These results confirm previous findings (Lemaire and Salette, 1984; Lemaire and Gastal, 1997). As a consequence of these trends in shoot N concentration and biomass accumulation, the NNI varied with the N fertilizer application rate (Figure 1). The NNI observed for the 120 and 60 N treatments were between 1·0 and 0·8. Even with the highest rate of N fertilizer application, similar to the level of mineral fertilization recommended by

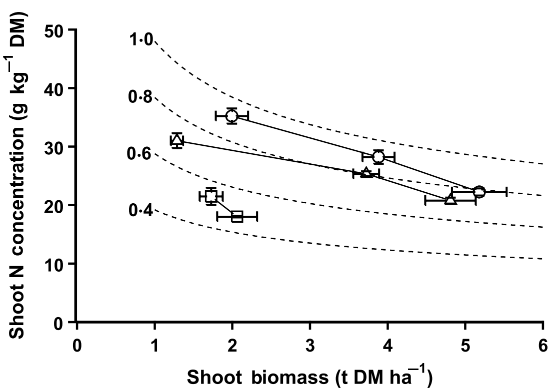

Figure 1 Effect of N fertilizer application rates [0 (□), 60 (△) and 120 (○) kg N ha^{-1}] on the relationship between shoot N concentration and biomass in Experiment 1 (spring). Errors bar is s.e. of mean. Dashed lines: sward Nitrogen Nutrition Index isolines.

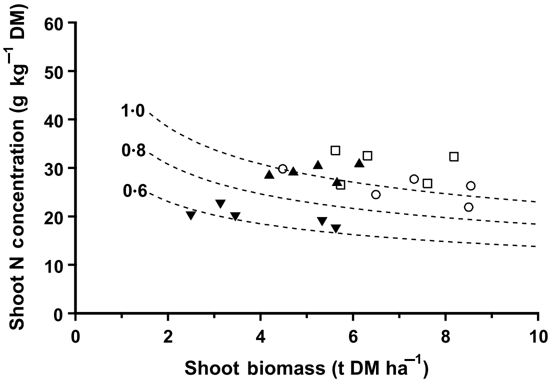

Figure 2 Effect of the N fertilizer management [conventional drained (○), conventional undrained (□), best nitrogen management practice undrained (▲) or 0 N rates (▼)] on the relationship between shoot N concentration and biomass in Experiment 2 (spring). Dashed lines: sward Nitrogen Nutrition Index isolines. Data points for individual quadrat measurements.

extension services in the UK, the NNI of the swards was not excessive. The NNIs, and hence the sward nitrogen status, were stable during the spring growth period for each N treatment, although they tended to decrease at the end of the growth period (third sampling date), particularly at the highest fertilizer application rate (120 N). This slight decline in NNI at the end of the growth period was likely to be due to a decreased availability of N in the soil since N was applied at the beginning of the growth period (April). Data obtained during the summer growth period showed similar ranges of NNI and similar trends and, therefore, are not shown.

Experiment 2

In Experiment 2, the sward nitrogen status also varied according to sward management (Figure 2). The large size of the plots and the presence of grazing animals may have induced a larger spatial heterogeneity in shoot N and biomass within a nitrogen fertilizer treatment than in Experiment 1 where plots were smaller and grazed at very low stocking rate at the end of the season. Therefore shoot N concentration and biomass data of each sampling are shown instead of average data per treatment as in Experiment 2. The NNI of the CONV/undrained plot was 1.16 on average, and thus appeared excessive. On the BNMP/undrained and on the CONV/drained plots, the NNI was satisfactory (close to 1.0 on average). On these three treatments, the NNI was higher than on the plots of Experiment 1 due to the higher N fertilizer application rates (120 kg N ha^{-1} for the highest rate of Experiment 1 compared with 170 kg N ha^{-1} for the CONV/undrained treatment of Experiment 2) and, in addition, the zero N treatment of Experiment 2 received manure in the previous autumn.

Relationships between Nitrogen Nutrition Index and N concentration of the upper leaves

Experiments 1 and 2

Using the data from both studies, the linear regression between NNI and N_{up} of the swards (Figure 3) showed a highly significant correlation ($r^2 = 0{\cdot}80$). The slopes and intercepts of the linear regressions between NNI and N_{up} did not differ statistically between the two experiments, despite substantial differences in fertilizer

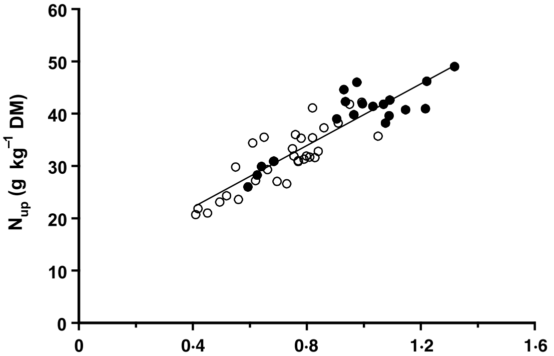

Figure 3 Relationship between Nitrogen Nutrition Index of the swards and N concentration of the lamina at the 10 cm top of the canopy (N_{up}) in Experiment 1 (○) and Experiment 2 (●). Data points for individual quadrat measurements. Continuous line: overall linear regression.

application rates, sward history and herbage utilization. The high correlation found on the permanent swards of the present study between NNI and N_{up} confirms preliminary findings obtained on pure stands of perennial ryegrass, tall fescue and cocksfoot under cutting management (Gastal *et al.*, 2001; Farruggia *et al.*, 2002). Therefore, the present data lead to the conclusion that, in order to evaluate the nitrogen nutritional status of permanent grass swards dominated by *H. lanatus*, *Lolium perenne* and *Agrostis* spp., the determination of the N concentration of upper leaves is a satisfactory alternative to the measurement of biomass and N concentration of the entire shoots.

The slope of the regression line obtained between NNI and N_{up} (g kg^{-1} DM) in the present study (NNI = 1·029 + 2·953 N_{up}) is slightly lower than that of the regression line obtained on pure stands of perennial ryegrass and tall fescue by Gastal *et al.* (2001). In the range of variation in NNI of 0·6–1·0, which represents the usual range of variation in NNI for grasslands, the relative difference in N_{up} between the two regression lines is no larger than 0·05. Considering all the possible sources of uncertainties in the determination of N concentration of herbage samples, it cannot be concluded that the two regression lines obtained in the present study and in the study from Gastal *et al.* (2001) are different.

As the swards of the present study had many species, the question of whether the dominant species, *H. lanatus* and perennial ryegrass, had a similar N concentration in the upper sward layer can be raised. A separate harvest and analysis of leaves by species indicated that the N concentration in the leaves of the upper sward layer was lower for *H. lanatus* than for perennial ryegrass (Figure 4). This was observed both in situations where the species were arranged in patches and in situations where they were more homogeneously mixed. This result was unexpected since earlier studies did not show significant differences in critical N concentration between multi-species permanent grasslands and monocultures (Duru *et al.*, 1997). *Holcus lanatus* is a species that has a higher specific leaf area than many other grasses (Boot, 1990). However, there is no indication that it could have an intrinsic lower N concentration than other grasses. Previous studies indicate that *H. lanatus* has a similar leaf N concentration to other fast-growing grasses when grown under non-limiting nitrogen supply, both in pot studies (Van der Werf *et al.*, 1993) and in field studies (P. Cruz, pers. comm.). Therefore, the lower N concentration, observed on the leaves of the upper sward layer of *H. lanatus* in the present study, may be determined by a lower ability to take up N than the other dominant species under situation of root competition, as occur in the dense mixed swards of the present study.

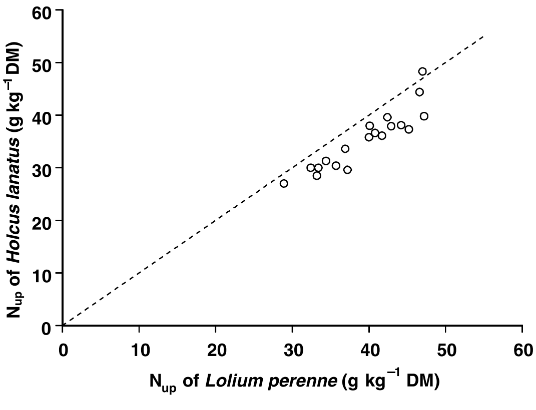

Figure 4 Relationship between N concentration of the lamina at the 10 cm top of the canopy (N_{up}) of the two main species of the swards (*Holcus lanatus* and *Lolium perenne*). Dashed line: 1:1 line.

Overall, the present results show the value of the N_{up} determination in evaluating sward N status (NNI). The determination of herbage biomass per unit area is no longer necessary, eliminating the most time-consuming step of the conventional NNI sampling procedure. However, the determination of N concentration on dried leaves remains to be done *a posteriori* in conventional analytical laboratories.

The N_{up} determination may also overcome some other limitations of the measurement of NNI. First, NNI measurement of individual species of mixed swards is problematic due to the uncertainties in evaluating biomass per unit area for each species (Cruz and Soussana, 1997). The N_{up} determination overcomes this difficulty. Secondly, the use of NNI also has limitations on continuously grazed swards due to the impact of continuous grazing on sward structure, on leaf:pseudostem ratio, and thus probably on critical N concentration. The N_{up} determination could overcome these uncertainties. However, it would be necessary to sample only ungrazed leaves in the top 10 cm, as there is a gradient in N concentration from the tip to the base of the leaves in grasses (Maurice, 1997). This requirement would probably restrict the use of the N_{up} determination to situations of lax continuous grazing.

Relationship between plant N status and soil mineral N

Experiments 1 and 2

The relationship between plant N status and soil mineral N was investigated using the data from both experiments. Using the entire data set, there was no

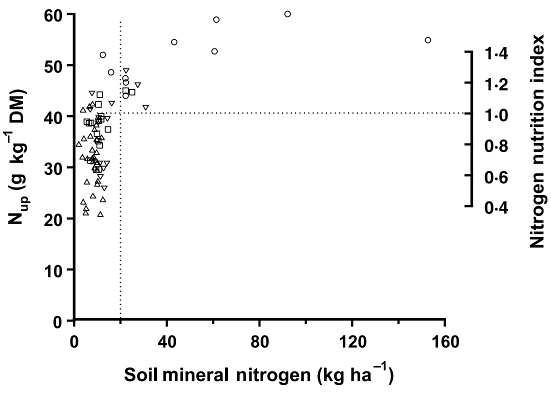

Figure 5 Relationship between soil mineral N content and N concentration of the lamina in the 10 cm top of the canopy (N_{up}) or Nitrogen Nutrition Index (NNI) for Experiment 1 (all dates, △) and Experiment 2 (11 March, □, 23 May, ▽ and 4 July, ○). Horizontal dotted line: isoline for NNI of 1·00. Vertical dotted line: isoline for soil mineral N content of 20 kg ha^{-1}.

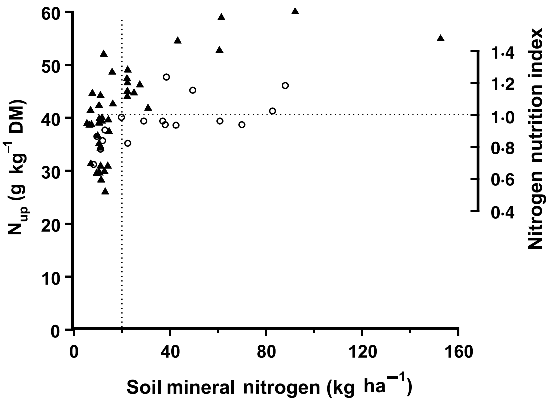

Figure 6 Relationship between soil mineral N and N concentration of the lamina in the 10 cm top of the canopy (N_{up}) and Nitrogen Nutrition Index (NNI) between spring (▲) and summer (○) data of Experiments 1 and 2. Horizontal dotted line: isoline for NNI of 1·00. Vertical dotted line: isoline for soil mineral N content of 20 kg ha^{-1}.

correlation between N_{up} and soil mineral N content within the range of 0–20 kg mineral N ha^{-1} (Figure 5). Within this range, N_{up} varied from 23 to 42 g kg^{-1} DM, corresponding to a range of 0.44–1.07 in NNI. Thus, sward nitrogen status varied from very limiting to no-limiting situations while soil mineral N content remained very low. Above a soil mineral N content of 20 kg ha^{-1}, a slight correlation was apparent with N_{up} with the overall data set. However, this slight correlation was restricted to situations of excessive NNI (N_{up} above 40 g kg^{-1} DM and thus an NNI of above 1·00).

Soil mineral N was significantly lower on the small plots in Experiment 1 than on the field-scale plots in Experiment 2. The small plots in Experiment 1 were characterized by a disturbed situation as they were obtained from an area previously unfertilized for 15 years and then subdivided into small plots fertilized at the onset of the experiment with several different mineral N fertilizer rates. In contrast, the large field-scale plots of Experiment 2 had operated for several years under several fertilizer-management regimes, which involved mineral inputs plus organic inputs, in the form of FYM and animal excreta. Thus the plots in the two experiments differed largely in composition and in duration of N inputs. The long-term and partly organic-N fertilizer applications to the field-scale plots of Experiment 2 probably led to a larger accumulation of soil organic N, and thus led to a larger potential N mineralization, than the small plots in Experiment 1. In the latter study, organic soil N content was probably lower, and, as a consequence, mineral N input was probably rapidly taken up either by the plants or immobilized by the microbial biomass.

In addition, a different relationship between soil mineral N content and N_{up}, and hence a different relationship between mineral N content and sward nitrogen status, was observed in the summer than in the spring (Figure 6). During the early summer period, a significant water shortage occurred. The low water content of the upper soil layer, where most of the root biomass and most of the mineral N are located, may have restricted plant N uptake, thus explaining the simultaneous occurrence of both soil mineral N accumulation and sward N limitation.

The simultaneous evaluation of both soil mineral N and sward N status conducted in the present study shows first that soil mineral N accumulates in substantial amounts when sward nitrogen index is above 1·00 (or N_{up} above 40 g kg^{-1} DM). This result is in line with current knowledge on other crops (Devienne-Barret *et al.*, 2000; Gastal and Lemaire, 2002). Thus under these circumstances, the two N indicators are in agreement as they both detect excessive N availability in the grassland system. Secondly, in the situations where N does not accumulate in excess with respect to sward N status (NNI below 1·00), the NNI may vary over a large range whereas soil mineral N content remains low. These situations may correspond to situations where the rate of sward N uptake is close to the rate of net N mineralization or, alternatively, to situations where sward N status is maintained due to prior plant N accumulation, and thus reflects past episodes of higher mineral soil N availability.

Conclusion

In grasslands, as in many other crops, NNI has proved to be a useful diagnostic tool of the N status of the vegetation. However, from a practical point of view, its determination is time-consuming due to the necessity of evaluating sward biomass per unit area, in addition to sampling herbage for N determination. The present study shows that determining the N concentration of lamina at the top of the canopy (N_{up}) provides a more practical and easier way to assess the nutritional status of a sward, as it does not require the determination of herbage biomass in addition to herbage N concentration. This determination also shows potential to overcome limitations to the use of NNI, in the case of mixed swards, and in a number of grazing situations. However under continuous grazing, the use of the N_{up} method should be more specifically evaluated. The relationship between N_{up} and NNI could also be valuable in remote-sensing methodologies where optical properties of the canopy are more dependant on properties related to N concentration at the top of the canopy than the entire above-ground herbage.

In the soil and management conditions in this study, NNI and soil mineral N content were poorly correlated. These two N diagnostic tools appear more related to particular soil or plant processes than to the overall efficiency of N use in grasslands. Therefore, their interest in terms of diagnosis appears to be rather objective-specific. Soil mineral N content is of interest in relation to the risk of nitrate leaching during potential drainage periods, and thus more relevant to environmental concerns, whereas sward nitrogen status is more relevant for the herbage production function of grasslands. As multifunctionality of grasslands is increasingly being considered (Hervieu, 2002), these two diagnostic tools should probably be considered as complementary tools, rather than being considered as concurrent tools, for monitoring, evaluation and prediction in grassland N management.

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