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Effects of cutting frequency on plant production, N-uptake and N_2 fixation in above- and below-ground plant biomass of perennial ryegrass–white clover swards

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Abstract

Nitrogen (N), accumulating in stubble, stolons and roots, is an important component in N balances in perennial ryegrass–white clover swards, and the effects of cutting frequency on the biomass of above- and below-harvest height were studied during two consecutive years. Total dry matter (DM) and total N production, and N_2 fixation, were measured at two cutting frequencies imposed in the summers of two years either by cutting infrequently at monthly intervals to simulate mowing or by frequent cutting at weekly intervals to simulate grazing. Total DM production harvested was in the range of 3000–7000 kg DM ha^{-1} with lower DM production associated with the frequent cutting treatment, and it was significantly affected by the different weather conditions in the two years. The higher cutting frequency also reduced the biomass below harvest height but the different weather conditions between years had less effect on stubble and, in particular, biomass of roots. The biomass of roots of white clover was significantly lower than that of roots of perennial ryegrass and remained at a relatively constant level (200–500 kg DM ha^{-1}) throughout the experiment, whereas the biomass of perennial ryegrass roots increased from 2400 kg DM ha^{-1} in the year of establishment to 10 200 kg DM ha^{-1} in the infrequent cutting treatment and 6650 kg DM ha^{-1} in the frequent cutting treatment by the end of the experiment, giving shoot:root ratios of 4·7–16·6 and 0·5–1·6 for white clover and perennial ryegrass respectively. Annual N_2 fixation was in the range of 28–214 kg N ha^{-1}, and the proportion of N fixed in stolons and roots was on average 0·28. However, as weather conditions affect the harvested DM production and the shoot:root ratio, care must be taken when estimating total N_2 fixation based on an assumed or fixed shoot:root ratio.

Keywords: field N balance, pasture, pNdfa, roots, shoot:root ratio, stolons

Introduction

The ability of clover to fix atmospheric nitrogen (N_2) is one of the main advantages when grown in mixture with grasses, and the process is fundamental for organic dairy farming which relies solely on this nitrogen (N) source. However, compared with mowing, grazing animals have profound effects on these legume-based pastures in several ways, including physical impact on soil and plants through treading, redistribution of nutrients through excreta and through more frequent defoliation (Menneer *et al.*, 2004).

N_2 fixation or the uptake of N in legume-based swards is often quantified on the basis of harvested plant material using a given shoot:root ratio, as for example by Kristensen *et al.* (1995) and Høgh-Jensen *et al.* (2004). From an environmental point of view, i.e. potential for N leaching, or from an agronomic point of view, i.e. residual effects on the succeeding crop, complete N budgets are necessary, including the contribution from the plant biomass below the harvested height. However, estimation of the total N_2 fixation, based on a given shoot:root ratio, can only be done providing that the shoot:root ratio is not affected by management, such as cutting frequency, or by environmental conditions.

It is known from several studies (Swift *et al.*, 1992; Elgersma and Schlepers, 1997; Schils *et al.*, 1998; Unkovich *et al.*, 1998) that cutting or grazing affects

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the amount of harvested biomass as well as the proportion of clover in grass–clover swards, and thereby also N_2 fixation. The distribution of biomass between harvested biomass and plant biomass below the harvested height, or between shoots and roots of clover and grass or grass-clover mixtures, has also been studied in pot and greenhouse experiments (Hakala *et al.*, 2005; Sturite *et al.*, 2005; Vinther, 2005) and in the field (Nassiri and Elgersma, 2002; Elgersma and Schlepers, 2005; Loges and Taube, 2005). However, not much is known about how cutting frequency or grazing affects the biomass of stubble and roots, and the shoot: root ratio.

The objective of this study was to investigate how cutting frequency under field conditions affects dry matter (DM) production, N uptake and N_2 fixation, with special focus on the plant biomass below harvest height, i.e. stubble, stolons and roots. Focusing on the plant biomass, parts of the study have in a summarized form been published together with results from greenhouse experiments, where effects of cutting on above- and below-ground biomass of two clover species were studied (Vinther, 2005). The present paper gives the full details of the field experiment.

Materials and methods

Establishment of sward

The field experiment was located at the Danish Institute of Agricultural Sciences, Research Centre Foulum, in the central part of Jutland, Denmark (9°34′E, 56°29′N). The soil is a sandy loam and is classified as a Typic Hapludult with 7–8% clay and 2–3% total carbon (C). The study was carried out during the growing seasons of 2002 and 2003 on a perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) sward which was established in 2001 by undersowing the perennial ryegrass and white clover in spring barley (*Hordeum vulgare* L.) sown on 9 April. On 15 April 2001, triplicate plots (4 m × 2 m) were labelled with ^{15}N [10 atm.% $(NH_4)_2SO_4$] in amounts corresponding to 1 g N m^{-2}, as described by Vinther and Jensen (2000), where ^{15}N was applied together with glucose as an immobilizing C source (8 g C m^{-2}) allowing ^{15}N to be incorporated in the soil microbial biomass. At the end of autumn 2001 (5 November), the plant material was harvested and stubble and root samples were taken in triplicate. The plant material was harvested by cutting the central area of the plots (1 m^2) to a height of *c.* 5 cm above the soil surface. Stubble and roots were sampled from each of the ^{15}N-labelled plots, where a steel cylinder (diameter 20 cm and height 20 cm) was inserted into the soil, removed and brought to the laboratory, and the plant material separated into perennial ryegrass stubble, white clover stubble including stolons, perennial ryegrass roots and white clover roots. Briefly, after cutting and separation of the above-harvest parts, the separation of white clover and perennial ryegrass roots was attempted by leaving the stubble as 'handgrip' and then gently separating the perennial ryegrass and white clover roots while rinsing off the soil with a shower. The proportion of roots, which could not be accounted for, was divided into white clover and perennial ryegrass roots using the white clover:perennial ryegrass ratio of the accounted-for root material. The proportion of unidentified roots varied between samples from 0·07 to 0·45 of the root biomass and was, as such, a significant source of uncertainty.

Treatments

After a first cut in late May 2002, each of the three 4 m × 2 m plots was split into two halves, each measuring 2 m × 2 m. One half was mowed, i.e. was cut three times in 2002 and four times in 2003 at approximately monthly intervals (infrequent cutting treatment), and the other half, simulating grazing, was cut seven times in 2002 and twelve times in 2003 at 8–14 d intervals (infrequent cutting treatment). Below-harvest height samples were taken four times during that period and treated as described above.

Determination of N_2 fixation

Symbiotic N_2 fixation was determined using the ^{15}N isotope dilution method (McAuliffe *et al.*, 1958; Fried and Middelboe, 1977). All the harvested or sampled plant material was, after separation into white clover and perennial ryegrass components, dried at 80°C overnight and weighed for determination of DM production. The dried material was then pulverized in a ball-mill for analysis of total N and ^{15}N. The concentrations of total N and ^{15}N were determined on a N-analyser coupled online to an isotope ratio mass spectrometer (Carlo Erba, Finnigan MAT; Carla Erba, Milan, Italy) as described by Jensen (1991). The proportion of N derived from the atmosphere (pNdfa) and N_2 fixation was calculated as described by Vinther and Jensen (2000), where the natural ^{15}N abundance of the soil was determined to be 0·3663 atm.% ^{15}N and the perennial ryegrass growing in mixture with the white clover was used as the reference plant:

$$\mathrm{pNdfa} = \frac{1 - \mathrm{atm.\%^{15}N\ excess(white\ clover)}}{\mathrm{atm.\%^{15}N\ excess(perennial\ ryegrass)}},$$

where atm.% ^{15}N excess = atm.% ^{15}N(white clover or perennial ryegrass) − 0·3663. The amount of N_2 fixed was then calculated as white clover DM × N concentration × pNdfa/100.

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Data analysis

The results in the figures and tables are presented as arithmetic mean ($n = 3$) with the standard error of mean (s.e.m.). Analysis of variance was carried out using the GLM procedure in SAS (SAS Institute Inc., 1989), which was used to estimate least significant differences at the $P < 0{\cdot}05$ significance level.

Results

Dry-matter production

In the year of establishment (2001) the total cumulated harvested biomass (s.e.m.), i.e. white clover plus perennial ryegrass, was 1 170 (120) kg DM ha^{-1} (Figure 1a). After initiation of the cutting experiment on 2 July 2002, the DM production of the infrequent and frequent cutting treatments gave a total cumulated yield of 7000 (350) and 5700 (380) kg DM ha^{-1} respectively (Figure 1a). The weather conditions in 2003 were very different from those of 2002. Average daily mean temperatures from February to April were 5·8°C in 2002 and 2·2°C in 2003, and precipitation during the summer months (June–August) was 100 mm lower in 2003 (272 mm) than in 2002 (172 mm). The cold spring and dry summer in 2003 was probably the reason for the considerably lower DM production than in 2002, with no significant difference between infrequent and frequent cutting treatments, resulting in total cumulated yields of 3240 (550) and 2970 (310) kg DM ha^{-1} respectively.

The proportion of white clover was about 0·40 of the total DM in the year of establishment, and decreased to about 0·30 at the first cut in May 2002 (Figure 1b). During the growing season of 2002 the proportion of white clover increased to about 0·70 followed by a decrease to 0·23–0·32 with no significant difference between infrequent and frequent cutting treatments. During most of the growing season of 2003 the proportion of white clover was significantly lower ($P < 0{\cdot}001$) in the infrequent than in the frequent cutting treatment but the difference slowly disappeared towards the end of the season (Figure 1b).

The biomasses of stubble and stolons were rather variable ranging from 400 kg DM ha^{-1} of white clover in the year of establishment to 1900 kg DM ha^{-1} of perennial ryegrass at the end of 2002, and there was no difference between infrequent and frequent cutting treatments, except for November 2003 when the biomass of stubble of perennial ryegrass was significantly ($P = 0{\cdot}03$) lower on the frequent cutting treatment than the infrequent cutting treatment (Figure 1c). The biomass of white clover roots was significantly lower than that of perennial ryegrass roots, and remained relatively low (200–500 kg DM ha^{-1}) during the entire period (Figure 1d). The biomass of perennial ryegrass roots increased from 2400 kg DM ha^{-1} in the year of establishment to 10 200 kg DM ha^{-1} in the infrequent cutting treatment and 6650 kg DM ha^{-1} in the frequent cutting treatment at the last sampling date in November 2003 (Figure 1d). There was no difference between infrequent and frequent cutting treatments on the biomasses of stubble and stolons, except in November 2003, when the biomass of perennial ryegrass roots was lower in the frequent cutting treatment than in the infrequent cutting treatment. The total biomasses of DM below harvest height, i.e. stubble and roots of perennial ryegrass plus stubble and roots of white clover, at the end of the growing season, were 9160 (2180) kg DM ha^{-1} and 7390 (1680) kg DM ha^{-1} on the infrequent and frequent cutting treatments, respectively, in 2002. In 2003, the biomasses of DM below harvest height were 12 170 (2700) kg DM ha^{-1} and 7890 (1930) kg DM ha^{-1} in the infrequent and frequent cutting treatments respectively. Thus, despite a considerably lower DM production in 2003 than in 2002, the biomass below harvest height remained at the same level in the two years, or even tended to increase between 2002 and 2003.

The time course of changes in stubble and root biomass is illustrated in Figure 2. For the sake of simplicity, average values between infrequent and frequent cutting treatments are presented showing that the maximum biomass of DM of white clover stubble (stolons) and roots was already reached at the end of the establishment year in 2001. The biomass of DM of perennial ryegrass stubble and roots continued to increase until the end of the experiment in the second production year of 2003. The increase in biomass of DM was primarily due to the increase in the biomass of DM of roots.

N_2 fixation and field N balances

Concentrations of N in harvested biomass, in stubble, stolons and in roots are shown in Figure 3. In the harvested biomass of white clover the concentration of N varied from 33 to 58 g kg^{-1} DM, and in the perennial ryegrass from 22 to 43 g kg^{-1} DM, and was, on average, 45 and 32 g kg^{-1} DM in white clover and perennial ryegrass respectively (Figure 3a). The concentration of N in the harvested biomass was, on average, during the two years significantly lower in the infrequent than in the frequent cutting treatment for both white clover ($P = 0{\cdot}03$) and perennial ryegrass ($P = 0{\cdot}02$). In stubble and roots the variation in concentration of N between treatments and harvest dates was relatively small (Figure 3b), and the mean concentrations of N of treatments and harvest dates for stubble and stolons

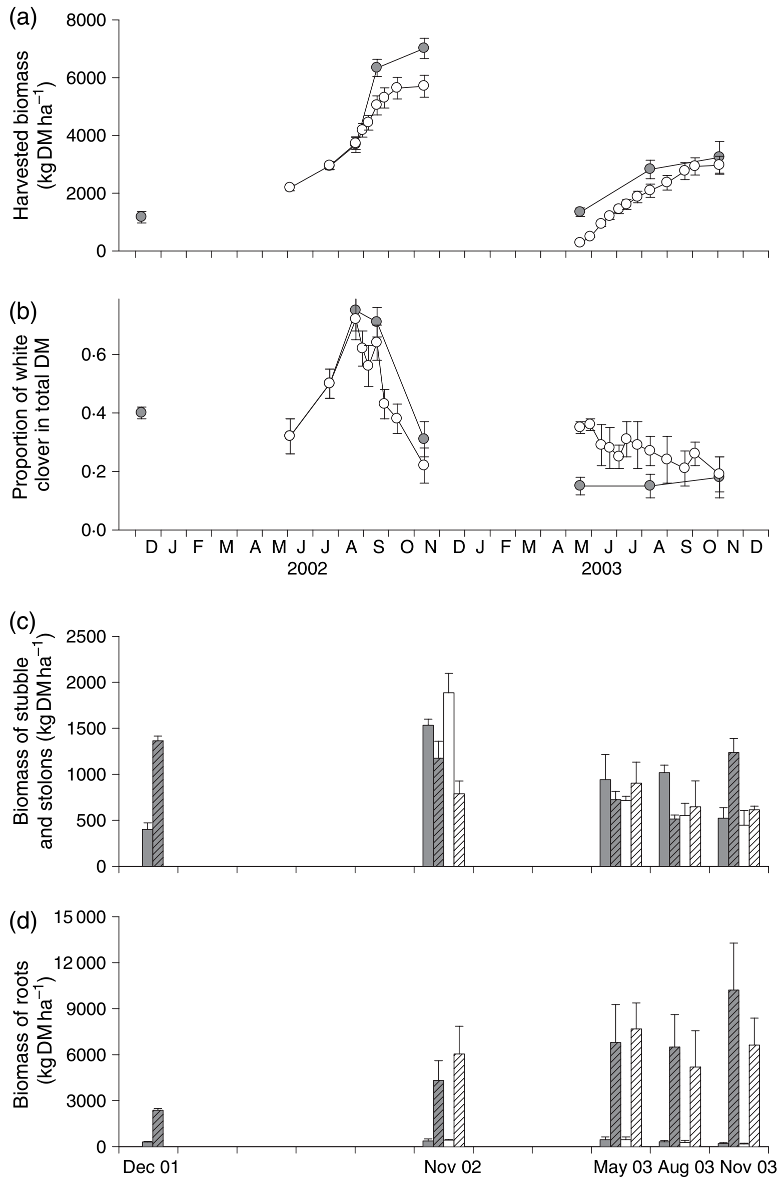

Figure 1 (a) Cumulated biomass of dry matter (DM), (b) harvested proportion of white clover in the harvested biomass, (c) biomass of DM of stubble and stolons and (d) biomass of DM of roots during the period from December 2001 to November 2003. In (c) and (d) dates are given as month and year. Grey symbols represent the infrequent cutting treatment and white symbols the frequent cutting treatment. Open and hatched bars represent white clover and perennial ryegrass respectively. Error bars are standard errors of the mean ($n = 3$).

were 31 g kg^{-1} DM in white clover and 20 g kg^{-1} DM in the perennial ryegrass stubble, and in white clover and perennial ryegrass roots 25 g kg^{-1} DM and 17 g kg^{-1} DM respectively (Figure 3c), and there was no significant difference between the infrequent- and the frequent-cutting treatments.

The pNdfa in the biomass of harvested white clover decreased during the experiment from 0·96 in 2001 to 0·74 and 0·60 at the last measuring date in 2003 in the infrequent- and frequent-cutting treatments respectively (Figure 4a). The pNdfa values were significantly lower ($P < 0{\cdot}01$) in the frequent-cutting treatment than in the infrequent cutting treatment. The pNdfa values in the harvested biomass were slightly higher than in stubble and stolons (Figure 4b), and pNdfa values in roots (Figure 4c) were slightly lower than in stubble and stolons but with no significant differences between treatments.

The annual N_2 fixation in the harvested biomass and in the total biomass, the latter including stubble, stolons and roots, is shown in Table 1. As a consequence of the relatively cold spring and dry summer in 2003, resulting in a low DM production of white clover, and the biased N_2 fixation caused by recycling of fixed N as discussed later, N_2 fixation was significantly lower in 2003 than in 2002. The fixation in 2002 was 131–171 kg N ha^{-1} in

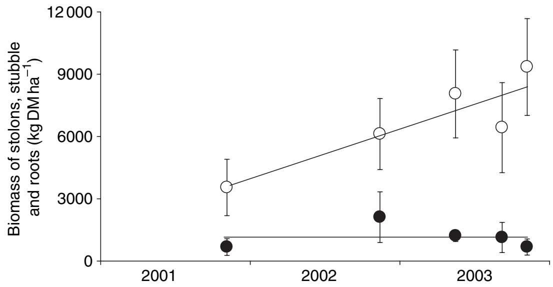

Figure 2 Time course of the biomass of dry matter (DM) below harvest height of white clover (closed symbols) and perennial ryegrass (open symbols) during the experiment from establishment (2001) until the end of the experiment (2003). Error bars are standard errors of the mean (n = 3).

the harvested biomass and 169–214 kg N ha^{-1} in the total biomass, and in 2003 only 15–27 and 28–38 kg N ha^{-1} respectively (Table 1).

Due to the climatic conditions and recycling of fixed N, the amount of N removed with the harvested biomass in 2003 was less than half of the amount removed in 2002, both in the frequent and in the frequent cutting treatments (Table 1). In all three years and in both treatments there was a negative field N balance, i.e. more N was removed with the harvested biomass than added through N_2 fixation, when only including N_2 fixation in the harvested biomass. By including the amount of N fixed in stolons and roots, the field N balances were less negative (Table 1).

Proportions in stubble and roots

The DM biomass below harvest height, and total N and fixed N, as a proportion of that in harvested DM, which were measured in the stubble and roots ($P_{\text{stubble+roots}}$), are shown in Table 2. Table 2 also includes data derived from Eriksen *et al.* (2004), where N_2 fixation was measured in perennial ryegrass/white clover swards of

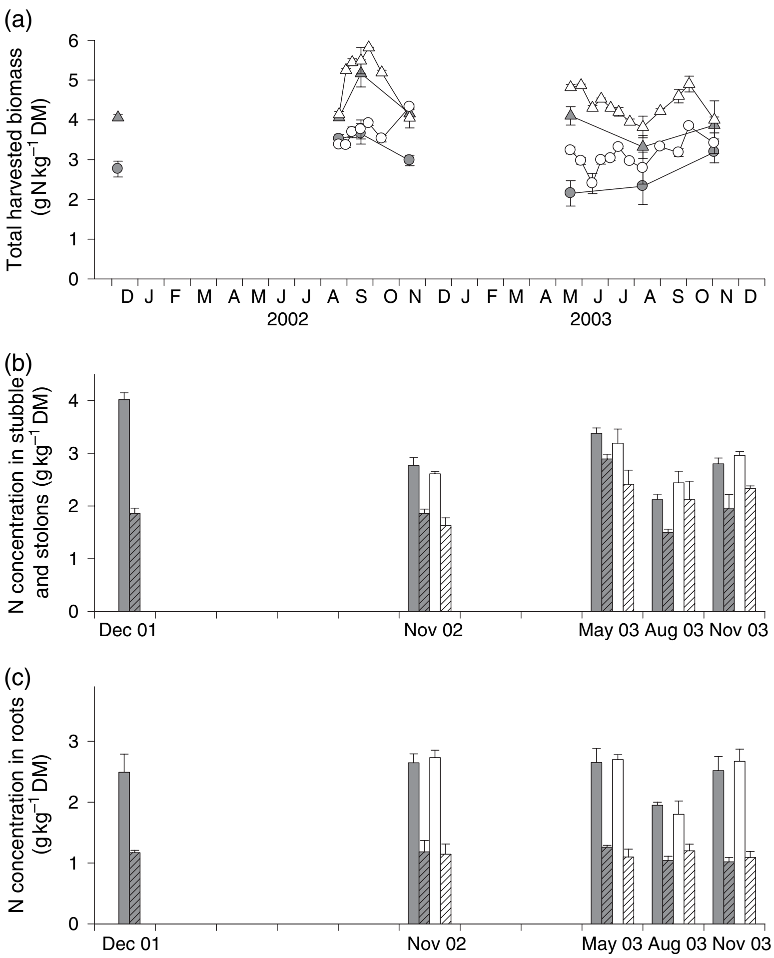

Figure 3 Concentration of nitrogen (N) in (a) total harvested biomass, (b) in stubble and stolons and (c) in the root biomass during the period from December 2001 to November 2003. In (b) and (c) dates are given as month and year. Grey symbols represent the infrequent cutting treatment and white symbols the frequent cutting treatment. Triangles and open bars represent white clover, and circles and hatched bars represent perennial ryegrass. Error bars are standard errors of the mean (n = 3).

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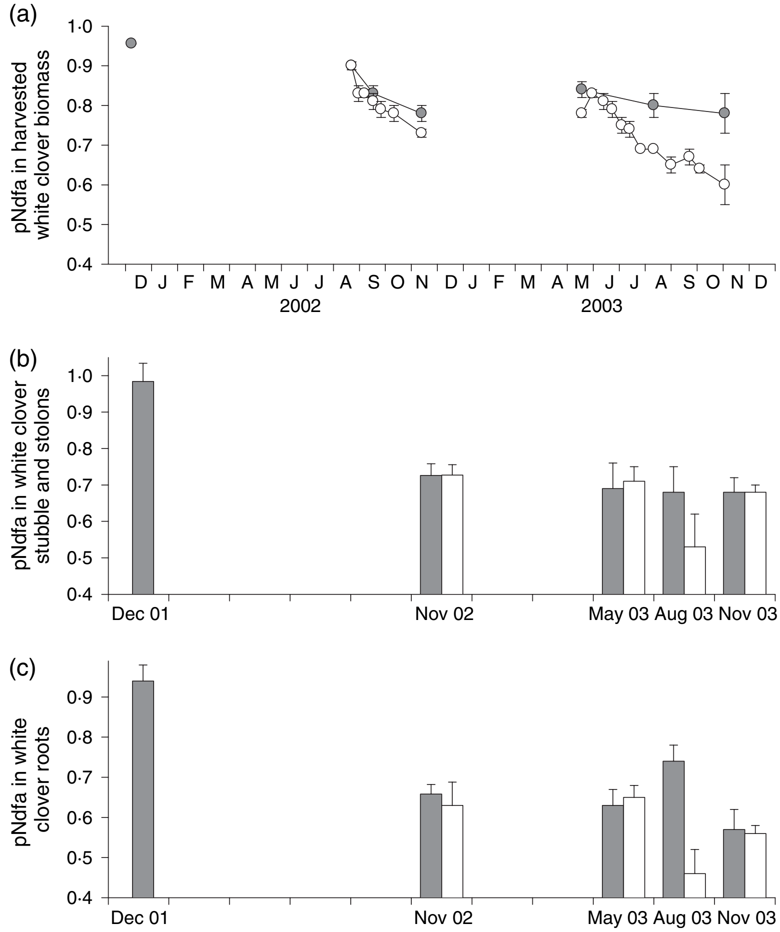

Figure 4 Proportion of N derived from the atmosphere (pNdfa) in (a) the harvested clover biomass, (b) in white clover stubble and stolons and (c) in white clover roots during the period from December 2001 to November 2003. In (b) and (c) dates are given as month and year. Grey symbols represent the infrequent cutting treatment and white symbols the frequent cutting treatment, and error bars are standard errors of the mean (n = 3).

different age using a procedure similar to that used in the present study. Total amounts, i.e. harvested plus below-harvest height biomass, can be calculated from the data in Table 2 as the harvested amount × (1 + $P_{stubble+roots}$). The $P_{stubble+roots}$ for white clover DM ranged from 0·4 to 1·4, whereas for perennial ryegrass $P_{stubble+roots}$ was in the range 1·1–4·2, showing that perennial ryegrass had a considerably larger below-harvest harvest biomass relative to white clover. For total N, the $P_{stubble+roots}$ ranged between 0·3 and 1·0 in white clover, and between 0·6 and 1·7 in perennial ryegrass, and, for N_2 fixation, $P_{stubble+roots}$ was in the range 0·26–0·88. There was no difference in $P_{stubble+roots}$ between cutting treatments.

The ratios between shoot and root biomass, where shoot includes stubble and stolons, showed significant differences between white clover and perennial ryegrass (Table 2). The shoot:root ratios of white clover were in the range 4·7–16·6 with an average of 8·7, whereas the range for perennial ryegrass was 0·5–1·6 with an average of 0·9. This shows that less than one-tenth of the above-ground biomass of white clover could be found in roots, whereas up to twice the above-ground biomass of perennial ryegrass may be found in the roots in the low-productive year of 2003.

Discussion

The weather conditions were quite different between the two years of the experiment, which had a significant influence on both DM production and N_2 fixation in the harvested biomass as well in stubble and roots, and, consequently, also on the distribution between the harvested and below-harvest height biomasses. Due to the variation in weather conditions, it is difficult to draw general conclusions concerning the direct effects of cutting frequency on harvested DM production. However, in the 'normal' year of 2002, frequent cutting, to simulate grazing, reduced the harvested DM biomass by 0·20 compared with infrequent cutting to simulate mowing. This is in agreement with earlier findings by Swift *et al.* (1992), who in a study with

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Table 1 Input of N by fixation, N removal by harvest and field N balance (kg N ha^{-1}) in the years 2001–2003 in infrequent and frequent cutting treatments. Data are presented as means (s.e.m.) (n = 3).

	2001	2002		2003	
		Infrequent	Frequent	Infrequent	Frequent
N_2 fixation in harvested biomass total biomass,	32 (6)	171 (15)	131 (1)	15 (3)	27 (7)
including stolons and roots	44 (8)	214 (13)	169 (13)	28 (5)	38 (10)
N removed with harvest					
White clover-N	34 (7)	205 (20)	162 (4)	20 (4)	37 (9)
Perennial ryegrass-N	34 (4)	82 (7)	92 (3)	76 (11)	66 (4)
Total N	68 (11)	287 (27)	255 (7)	95 (15)	103 (13)
N in biomass below harvest height					
White clover N	11 (3)	61 (8)	52 (3)	20 (5)	18 (5)
Perennial ryegrass N	25 (1)	76 (9)	68 (9)	128 (20)	87 (21)
Total N	36 (4)	137 (17)	120 (12)	147 (25)	105 (26)
Field N balance*	−36 (17)	−116 (42)	−124 (8)	−80 (18)	−76 (20)
Field N balance†	−24 (19)	−73 (40)	−86 (10)	−67 (20)	−65 (23)

*N_2 fixation in harvested clover biomass minus total N removed with harvest.
†N_2 fixation in total clover biomass, including stolons and roots minus total N removed with harvest.

Table 2 Biomass of dry matter (DM) below harvest height, and total N and N_2 fixation, as proportion of that in harvested DM ($P_{stubble+roots}$), and shoot:root ratios of white clover (WC) and perennial ryegrass (RG) in mixed sward. Data are means (s.e.m.) (n = 3).

	DM		Total N		N_2 fixation	Shoot:root ratio of DM	
Treatment	WC	RG	WC	RG	WC	WC	RG
Mowed (2001)*	0·4 (0·1)	1·5 (0·3)	0·3 (0·1)	0·8 (0·1)	0·35 (0·06)	6·8 (1·4)	1·6 (0·2)
Infrequent cutting (2002)	0·5 (0·1)	2·8 (0·6)	0·3 (0·1)	0·9 (0·1)	0·26 (0·04)	15·2 (0·8)	0·6 (0·2)
Infrequent cutting (2003)	1·4 (0·2)	4·2 (0·9)	1·0 (0·2)	1·7 (0·1)	0·88 (0·23)	5·6 (0·6)	0·5 (0·1)
Frequent cutting (2002)	0·6 (0·1)	2·2 (0·6)	0·3 (0·1)	0·7 (0·1)	0·28 (0·01)	16·6 (5·7)	1·0 (0·2)
Frequent cutting (2003)	0·7 (0·1)	3·5 (0·9)	0·5 (0·1)	1·4 (0·4)	0·42 (0·01)	7·0 (0·5)	0·5 (0·1)
From Eriksen *et al.* (2004)							
Mowed, year 1	0·6 (0·2)	1·1 (0·3)	0·5 (0·1)	0·6 (0·1)	0·38 (0·11)	5·8 (1·5)	1·5 (0·3)
Mowed, year 2	0·5 (0·1)	1·7 (0·2)	0·3 (0·1)	0·7 (0·1)	0·27 (0·05)	7·9 (2·1)	0·7 (0·1)
Mowed, year 8	0·7 (0·2)	2·2 (0·4)	0·5 (0·1)	1·1 (0·1)	0·40 (0·09)	4·7 (1·5)	0·7 (0·2)

*Year of establishment.

different white clover varieties found up to a 0·50 reduction in DM yield when comparing simulated grazing with five to six cuts annually. Similarly, Unkovich *et al.* (1998) found a 0·27 reduction in the total DM production of an intensively grazed sward compared with a lightly grazed sward, and Elgersma and Schlepers (1997) measured a 0·11–0·12 lower DM production in grass–clover swards cut more frequently (at a herbage mass of 1200 kg DM ha^{-1}) than when cut when the herbage mass reached 2000 kg DM ha^{-1}.

The variation in weather conditions had the most significant effect on the harvested biomass, which in the 'abnormal' year of 2003, with a cold spring and a dry summer, was about half of that in the 'normal' year of 2002. On the other hand, the biomass below harvest height was more or less unaffected by the variation in weather conditions between 2002 and 2003, with a constant increase over time. However, this increase was caused by an increase in the biomass of perennial ryegrass roots only, as the biomass of white clover roots remained more or less constant over time. Similarly, Eriksen *et al.* (2004) found no difference between the biomass of white clover below harvest height in 1-, 2- and 8-year-old pastures (*c.* 1000 kg DM ha^{-1}), whereas the biomass of perennial ryegrass below harvest height increased from about 4000 kg DM ha^{-1} in year 1 to about 9000 kg DM ha^{-1} in year 8.

The concentrations of N in harvested biomass found in the present study were very similar to those reported by Hartwig *et al.* (2000), who found average values at 28, 22 and 12 g N kg^{-1} DM in the harvested grass biomass, grass stubble and roots, respectively, and 43, 29 and 29 g N kg^{-1} DM in the harvested clover biomass, stolons and roots respectively. Nodules on white clover roots were not counted in the present study, but during washing it was observed that the roots were well nodulated, which may explain the higher concentrations of N in white clover than in perennial grass roots.

The use of perennial ryegrass grown in a mixture with white clover as the reference plant may be criticized. It has been proposed that, if the reference crop is grown in a mixture with the N_2-fixing crop, the pNdfa values may be underestimated (McNeill and Wood, 1990), because of transfer of fixed N from clover to grass, whereby the enrichment in the grass becomes lower than in the soil or lower than when grown in separate plots. Therefore, the decrease in pNdfa over time may not solely be caused by an increase in soil N by age of the pasture (Eriksen *et al.*, 2004) but may also be biased by an increasing proportion of grass-N originating from transfer of fixed N with a lower enrichment. It is generally accepted that pNdfa is high, ranging between 0·85 and 0·95, in grass–clover mixtures, due to the strong competitiveness of the grass in the uptake of soil nitrogen (e.g. Ledgard and Steele, 1992). Consequently, clover is more dependent on symbiotic N_2 fixation than that grown in monoculture, where pNdfa generally is in the range of 0·6–0·8 (Seresinhe *et al.*, 1994). In the present study pNdfa values in the harvested biomass were in the range of 0·78–0·96 in the infrequent-cutting treatment, whereas pNdfa values in the frequent-cutting treatment decreased from 0·83 to 0·60. These significantly lower values, corresponding to values in a monoculture of white clover, are presumably a result of lower DM production and consequently less N uptake by the perennial ryegrass when frequently cut, whereby more soil N was available and white clover was less dependent on atmospheric-N. Furthermore, frequent cutting increases the production of root exudates (Tisdall and Oades, 1979) and reduces the proportion of fine roots (Menneer *et al.*, 2004), which also increases the availability of N in the soil, and thereby reduces the dependency on atmospheric-N.

While the different weather conditions caused a large variation in white clover production and annual N_2 fixation rates, as also observed by Vinther and Jensen (2000) and Carlsson and Huss-Danell (2003), there is usually a significant association between proportion of legume in the sward and the amount of N fixed (Bowman *et al.*, 2004). In the present study, the amount of N fixed in the harvested biomass was in the range of 29–42 kg N t^{-1} harvested DM of white clover, which corresponds with earlier reports, as reviewed by Carlsson and Huss-Danell (2003), who, on average, found that the amount of N fixed in clover corresponded to 31 kg N t^{-1} harvested DM of clover. The amount of N fixed in the total biomass, including stolons and macro-roots, was in the range of 44–68 kg N t^{-1} harvested DM of white clover, which corresponds to the range of 51–65 kg N t^{-1} harvested DM of white clover reported by Elgersma and Schlepers (1997) and Elgersma *et al.* (1998).

Effects of cutting frequency on $P_{\text{stubble+roots}}$ and shoot:root ratios were, to some extent, overridden by variations in weather conditions between years, and no significant difference between infrequent and frequent cutting treatments was found. However, if values in 2003 are excluded, the average $P_{\text{stubble+roots}}$ for N_2 fixation was 0·28, which is slightly higher than the 0·25 suggested for the empirical N_2 fixation model by Høgh-Jensen *et al.* (2004). However, the results also show that weather conditions have a significant influence on $P_{\text{stubble+roots}}$ and the shoot:root ratio of white clover in particular, indicating that white clover is more sensitive to the weather conditions encountered than perennial ryegrass. Based on short-term field and pot experiments with leaf ^{15}N-labelling of white clover plants, Jørgensen and Ledgard (1997) suggested a $P_{\text{stubble+roots}}$ of 0·7 for calculating total N_2 fixation, and from Bouchart *et al.* (1998) a $P_{\text{stubble+roots}}$ at 0·7 can be calculated; both values are somewhat higher than that found in the present study. However, short-term studies give a lower harvested biomass and consequently a higher $P_{\text{stubble+roots}}$ than, for example, in the normal year (2002) of the present study. In the abnormal year (2003) with lower DM production of white clover, the $P_{\text{stubble+roots}}$ were 0·88 and 0·42 in the infrequent- and frequent-cutting treatments, respectively, which corresponds fairly well with the value of 0·7 found by Jørgensen and Ledgard (1997) in a short-term study. From Jørgensen and Ledgard (1997) a white clover shoot:root ratio of *c.* 4·0 can be estimated and a shoot:root ratio of 3·0 in subterranean clover can be derived from the data of McNeill *et al.* (1997), who also used a leaf-labelling technique. Similar to the present study, Callow *et al.* (2005) found in pot and small plot experiments that the root biomass of perennial ryegrass constituted a considerable proportion of the total plant biomass with shoot:root ratios estimated to be in the range of 0·8–1·2. Furthermore, in a comparison among barley, oats, rape and annual ryegrass, Pietola and Alakukku (2005) found that the root biomass of annual ryegrass was the greatest, and Zagal (1994) found, after a 65-d growing period in growth chambers, a shoot:root ratio at 0·8 for perennial ryegrass. In summary, the results of the present study clearly demonstrate the importance of including the below-harvest height fraction ($P_{stubble+roots}$) for estimating total N_2 fixation, but it must be underscored that a fixed shoot:root ratio must be used cautiously.

Even by including the amount of N fixed in stolons and roots, negative field N-balances were obtained, i.e. more N was removed with the harvested biomass than added through N_2 fixation. However, by further including estimates of the proportion of fixed N, which was transferred from white clover to perennial grass ($P_{trans} = 0{\cdot}2 \times$ harvested) and the proportion of fixed N immobilized in the soil ($P_{immobile} = 0{\cdot}25 \times$ harvested) as proposed by Høgh-Jensen *et al.* (2004), the field N balances came close to neutral in 2002, but were still negative in 2003 (not shown). This supports earlier findings by Thomas (1992) who, based on simulations, concluded that a range of fixation of 120–352 kg N ha^{-1} $year^{-1}$ is required to contribute to productive and sustainable pasture systems.

Conclusions

The results of the present study indicate that, compared with a normally mowed grass–clover sward, frequent cutting, simulating grazing, reduced both the harvested and below-harvest height DM production but resulted in shoot:root ratios with no significant differences between the two cutting strategies. Furthermore, the results showed that weather conditions have a significant effect on harvested DM production of grass–clover swards, whereas the below-harvest height DM production, i.e. stubble and roots, was less affected. The effects of weather were more important in white clover than in perennial ryegrass, resulting in a relatively high shoot:-root ratio under weather conditions giving normal to high annual DM production, and a low shoot:root ratio under weather conditions giving low annual DM production. Thus, when estimating the total fixed N_2, a fixed shoot:root ratio must be used cautiously taking into account the growth conditions and DM production in the actual year.

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References

Bouchart V., Macduff J.H., Ourry A., Svenning M.M., Gay A.P., Simon J.C. and Boucaud J. (1998) Seasonal pattern of accumulation and effects of low temperatures on storage compounds in *Trifolium repens*. *Physiologia Plantarum*, **104**, 65–74.

Bowman A.M., Smith W., Peoples M.B. and Brockwell J. (2004) Survey of the productivity, composition and estimated inputs of fixed nitrogen by pastures in central-western New South Wales. *Australian Journal of Experimental Agriculture*, **44**, 1165–1175.

Callow M.N., Michell P., Baker J.E., Cocks P.S. and Hough G.M. (2005) Response of herbage regrowth and water-soluble carbohydrate concentration of ryegrass species to defoliation practices when grown in a Mediterranean environment. *Grass and Forage Science*, **60**, 59–67.

Carlsson G. and Huss-Danell K. (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil*, **253**, 353–372.

Elgersma A. and Schlepers H. (1997) Performance of white clover/perennial ryegrass mixtures under cutting. *Grass and Forage Science*, **52**, 134–146.

Elgersma A. and Schlepers H. (2005) Weed interactions in establishing pure and mixed grass-clover swards; effects of clover and grass variety on root and shoot competition. In: Frankow-Lindberg B.E. (ed.) *Adaptation and management of forage legumes – strategies for improved reliability in mixed swards. Proceedings of the 1st COST-852 Workshop, Ystad, Sweden, 2004*, pp. 274–277.

Elgersma A., Nassiri M. and Schlepers H. (1998) Competition in perennial ryegrass–white clover mixtures under cutting. 1. Dry-matter yield, species composition and nitrogen fixation. *Grass and Forage Science*, **53**, 353–366.

Eriksen J., Vinther F.P. and Søegaard K. (2004) Nitrate leaching and N_2 fixation in grasslands of different composition, age and management. *Journal of Agricultural Science, Cambridge*, **142**, 141–151.

Fried M. and Middelboe V. (1977) Measurement of amount of nitrogen fixed by a legume crop. *Plant and Soil*, **47**, 713–715.

Hakala K., Nikunen H.-M. and Jauhiainen L. (2005) Biomass production in different grass/red clover mixtures. In: Frankow-Lindberg B.E. (ed.) *Adaptation and management of forage legumes – strategies for improved reliability in mixed swards. Proceedings of the 1st COST-852 Workshop, Ystad, Sweden, 2004*, pp. 183–186.

Hartwig U.A., Lüscher A., Daepp M., Blum H., Soussana J.F. and Nösberger J. (2000) Due to symbiotic N_2 fixation, five years of elevated atmospheric p_{CO2} had no effect on the N concentration of plant litter in fertile, mixed grassland. *Plant and Soil*, **224**, 43–50.

Høgh-Jensen H., Loges R., Jorgensen F.V., Vinther F.P. and Jensen E.S. (2004) An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. *Agricultural Systems*, **82**, 181–194.

Jensen E.S. (1991) Evaluation of automated analysis of ^{15}N and total N in plant material and soil. *Plant and Soil*, **133**, 83–92.

Jørgensen F.V. and Ledgard S.F. (1997) Contribution from stolons and roots to estimates of the total amount of N2 fixed by white clover (*Trifolium repens* L.). *Annals of Botany*, **80**, 641–648.

KRISTENSEN E.S., HOGH-JENSEN H. and KRISTENSEN I.S. (1995) A simple model for estimation of atmospherically-derived nitrogen in grass-clover systems. *Biological Agriculture and Horticulture*, **12**, 263–276.

LEDGARD S.F. and STEELE K.W. (1992) Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil*, **141**, 137–153.

LOGES R. and TAUBE F. (2005) Yield, forage quality, residue nitrogen and nitrogen fixation of different forages legumes. In: Frankow-Lindberg B.E. (ed.) *Adaptation and management of forage legumes – strategies for improved reliability in mixed swards. Proceedings of the 1st COST-852 Workshop, Ystad, Sweden, 2004*, pp. 129–132.

MCAULIFFE C., CHAMBLEE D.S., URIBE-ARANGO H. and WOODHOUSE W.W. JR (1958) Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by 15N. *Agronomy Journal*, **50**, 334–337.

MCNEILL A.M. and WOOD M. (1990) ^{15}N estimates of nitrogen fixation by white clover (*Trifolium repens* L.) growing in a mixture with ryegrass (*Lolium perenne* L.). *Plant and Soil*, **128**, 265–273.

MCNEILL A.M., ZHU C.Y. and FILLERY I.R.P. (1997) Use of *in situ* ^{15}N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil-plant systems. *Australian Journal of Agricultural Research*, **48**, 295–304.

MENNEER J.C., LEDGARD S., MCLAY C. and SILVESTER W.W. (2004) The impact of grazing animals on N_2 fixation in legume-based pastures and management options for improvement. *Advances in Agronomy*, **83**, 181–241.

NASSIRI M. and ELGERSMA A. (2002) Effects of nitrogen on leaves, dry matter allocation and regrowth dynamics in *Trifolium repens* L. and *Lolium perenne* L. in pure and mixed swards. *Plant and Soil*, **246**, 107–121.

PIETOLA L. and ALAKUKKU L. (2005) Root growth dynamics and biomass input by Nordic annual field crops. *Agriculture, Ecosystems and Environment*, **108**, 135–144.

SAS Institute Inc. (1989) *SAS/STAT Guide for Personal Computers*, Version 6, pp. 549–640. Cary, NC: SAS Institute Inc.

SCHILS R.L.M., VELLINGA TH.V. and KRAAK T. (1998) Dry-matter yield and herbage quality of a perennial ryegrass/white clover sward in a rotational grazing and cutting system. *Grass and Forage Science*, **54**, 19–29.

SERESINHE T., HARTWIG U.A., KESSLER W. and NOSBERGER J. (1994) Symbiotic nitrogen fixation of white clover in a mixed sward is not limited by height of repeated cutting. *Journal of Agronomy and Crop Science*, **172**, 279–288.

STURITE I., HENRIKSEN T.M., JØRGENSEN M. and VILEID M. (2005) Nitrogen accumulation in white clover and losses during winter. In: Frankow-Lindberg B.E. (ed.) *Adaptation and management of forage legumes – strategies for improved reliability in mixed swards. Proceedings of the 1st COST-852 Workshop, Ystad, Sweden, 2004*, pp. 125–128.

SWIFT G., MORRISON M.W., CLELAND A.T., SMITH-TAYLOR C.A.B. and DICKSON J.M. (1992) Comparison of white clover varieties under cutting and grazing. *Grass and Forage Science*, **47**, 8–13.

THOMAS R.J. (1992) The role of the legume in the nitrogen cycle of productive and sustainable pastures. *Grass and Forage Science*, **47**, 133–142.

TISDALL J.M. and OADES J.M. (1979) Stabilization of soil aggregates by the root system of ryegrass. *Australian Journal of Soil Research*, **17**, 429–441.

UNKOVICH M., SANFORD P., PATE J. and HYDER M. (1998) Effects of grazing on plant and soil nitrogen relations of pasture-crop rotations. *Australian Journal of Agricultural Research*, **49**, 475–485.

VINTHER F.P. (2005) Below-harvest biomass in grass-clover at different cutting frequencies. In: Frankow-Lindberg B.E. (ed.) *Adaptation and management of forage legumes – strategies for improved reliability in mixed swards. Proceedings of the 1st COST-852 Workshop, Ystad, Sweden, 2004*, pp. 187–190.

VINTHER F.P. and JENSEN E.S. (2000) Estimating legume N_2 fixation in grass-legume mixtures of a grazed organic cropping system using two ^{15}N methods. *Agriculture, Ecosystems and Environment*, **78**, 139–147.

ZAGAL E. (1994) Carbon distribution and nitrogen partitioning in a soil-plant system with barley (*Hordeum vulgare* L.), ryegrass (*Lolium perenne* L.) and rape (*Brassica napus* L.) grown in a $^{14}CO_2$-atmosphere. *Plant and Soil*, **166**, 63–74.

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