Longevity of White Clover (Trifolium repens) Leaves, Stolons and Roots, and Consequences for Nitrogen Dynamics under Northern Temperate Climatic Conditions

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• Background and Aims White clover (Trifolium repens) is, due to nitrogen (N) fixation, important to the N dynamics of several northern temperate agroecosystems. This study aimed at monitoring growth and death of major white clover plant organs to assess their potential contribution to within-season N input and risk of off-season N losses.

• Methods White clover (‘Snowy’) was studied in a plot and root window experiment in southeast Norway (60°42’N, 10°51’E). Leaves, stolons and roots were tagged for lifespan measurement in harvested and unharvested stands during two experimental years. The availability of soil inorganic N was measured by plant root simulator (PRS™) probes.

• Key Results The longevity of leaves and petioles ranged from 21 to 86 d (mean = 59 d), of main stolon sections from 111 to over 677 d (mean = 411 d) and of roots from 27 to 621 d (mean = 290 d). About 60% of the leaves produced had turned over by the end of the growing season and another 30% had died or disappeared by the subsequent spring. Harvesting reduced the longevity of stolons and increased plant fragmentation, but did not decrease leaf or root lifespan or increase soil N availability. From the plant organ turnover data, it was estimated that the gross N input to the soil–plant system from white clover in pure stand during two growing seasons corresponded to a 2.5-fold increase over the total N in harvestable shoots.

• Conclusions The short lifespan and poor over-wintering of leaves showed their potential importance as a nitrogen source in the soil–plant ecosystem but also their potential contribution to the risk of off-season N losses.

Key words: Harvesting, inorganic nitrogen, lifespan, leaves, main stolons, roots, Trifolium repens, turnover, white clover.

INTRODUCTION

In plant communities including white clover (Trifolium repens), it is important for production (Berry et al., 2002) and environmental reasons (Hansen et al., 2000) that symbiotically fixed nitrogen be carried over within the ecosystem to the subsequent season. In a recent study in Norway, Sturite et al. (2006) found that white clover leaves lost between 57 and 74% of their N between autumn and the following spring. In England, where the winter climate is milder, Woledge et al. (1990) similarly found that two-thirds of the leaf lamina weight of white clover was lost during the winter. Thus, factors in addition to temperature seem to influence leaf turnover during the winter. One explanation could be that leaf lifespan is inherently short (Brougham, 1958; Turkington, 1983) and that leaves generally do not live long enough to survive the cold season under northern conditions.

Consequently, stolons and roots seem to be the major N storage organs over the winter. Despite numerous studies on stolon characteristics (Hay, 1983; Davies and Jones, 1992) and winter survival (Collins et al., 1991; Collins and Rhodes, 1995; Frankow-Lindberg and von Fircks, 1998; Sturite et al., 2006), there are few data on the longevity of stolons under northern climate conditions. Data on the lifespan of white clover roots are also limited. Sturite et al. (2006) found that roots seemed to live longer than leaves. Watson et al. (2000), on the other hand, found that root turnover was quite rapid and more so in a warmer (Italy) than in a cooler climate (UK).

Cutting or grazing results in an allocation of resources from stolons and roots to leaf re-growth (Marriott and Haystead, 1990; Sturite et al., 2006), which may result in reduced root elongation and increased root death (Evans, 1973). Nitrogen uptake from soil decreases within a few hours after harvest for both forage grass and legumes, remains negligible for 7–9 d (Clement et al., 1978; Volenc et al., 1996) and may even result in net N efflux, as observed both for timothy (Phleum pratense) and meadow fescue (Festuca pratensis) (Bakken et al., 1998).

Thus, after harvest, one could expect a temporary increase in soil inorganic N as a result of N mineralization from dying roots, damaged aboveground plant material and soil organic matter.

In the present study, the longevity of white clover plant organs was investigated with a focus on consequences for N dynamics with regard to plant growth and environmental aspects. The hypotheses were that white clover leaves have a short lifespan and generally do not preserve N as well as
over the winter as stolons and roots, that turnover of plant organs correlates with cumulative temperature after emergence and that harvesting reduces the stolon and root longevity and temporarily increases soil inorganic N.

MATERIALS AND METHODS

Experimental site

A combined plot and root window experiment was conducted at Apelsvoll Research Centre in southeast Norway (60°42′N, 10°51′E), where the climate is typical for inland Scandinavia. Winters are relatively cold with snow-covered ground from mid October to mid April, and summers are warm and somewhat dry. Temperature and precipitation data for the experimental period (Fig. 1) were taken from a local weather station 150 m from the experimental site. The soil on the site is a morainic loamy sand (6 % organic matter, 44 % sand, 35 % silt and 21 % clay).

Establishment of root windows

Three metal frames (300 cm × 30 cm), divided into 12 sections (25 cm × 30 cm each), were mounted at an angle of 54° with the horizontal plane on wooden boxes (300 cm long, 90 cm wide and 90 cm deep). Trenches were dug out and the wooden boxes were placed therein, before three Plexiglas™ panes (100 cm × 30 cm × 0.3 cm) were installed on each frame. The excavated topsoil was refilled into the trenches and pressed carefully against the Plexiglas™ panes (Fig. 2). The effective soil depth of the root windows was 25 cm. The observation trenches were covered with a removable plywood sheet and 10 cm insulation mats to protect roots near the windows from unrealistic temperature and light conditions.

Plant material

In 2002, a genetically homogeneous white clover Trifolium repens L. clone of the cultivar ‘Snowy’, which is bred for northern temperate conditions and therefore quite winter hardy (Rapp, 1996), was produced by cutting stolon pieces of two or three nodes each. The pieces were planted in peat soil and placed in a greenhouse (18 °C; 24 h daylight). After 4 weeks of growth, the genetically identical clonal fragments (later called parent fragments) were placed at 6 °C for 10 d for acclimation before being transferred to the experimental site. Meadow fescue Festuca pratensis L. ‘Fure’) plants were established from seeds and grown in an agricultural soil (6 % organic matter, 44 % sand, 35 % silt and 21 % clay) under controlled temperature and light conditions (18 °C; 12 h 48 μmol m−2 s−1 from high pressure sodium lamps) for 6 weeks before they were planted on the experimental site.

Experimental design

The experiment lasted from June 2002 until May 2004. On 7 June 2002, three field plots of 1 m² each were established along each of the three window frames, which represented blocks of replicate treatments (Fig. 2). The plots within blocks were separated by 20 cm × 3 cm boards. Four white clover plants were planted on each 1 m² plot. On one of these plots, 40 meadow fescue plants were planted in mixture with the white clover. The plots were fertilized with 44 kg N, 20 kg P and 72 kg K ha⁻¹. During the first experimental year, one white clover plot in each block was left undisturbed, while the other two plots were harvested three times (4 cm cutting height). In the second experimental year, the same harvesting regime was applied, except that the mixed clover–fescue plots were split, and half of the plot was left undisturbed. Elongation of white clover stolons along the wooden boards was regulated manually and stolons forced to grow back into the plot in order to reduce edge effects between the plots.

Stolon and leaf observations

Four days after planting, two main stolons on each white clover plant were chosen randomly for monitoring and
marked with a white plastic tag at the terminal bud, which was at the stage of poorly developed adjacent leaves [between 0-2 and 0-3 on the scale proposed by Carlson (1966)]. The chosen stolons were then tagged at the bud every second week until mid October. The procedure was resumed in April 2003, just after snowmelt, and the plants were tagged and monitored every 4 weeks until the end of October. The final assessment was performed in April 2004. Stolon colour and physical appearance were visually assessed between tags (hereafter called stolon sections). Dark or light brown and soft stolons were assumed to be dead. On the same occasions, young but fully developed white clover leaves [between 0-9 and 1-0 on the Carlson (1966) scale] next to the stolon tags were also assessed. Dark brown or missing leaves were assumed to be dead. On harvested plots, petioles left after harvesting were assessed accordingly. On plots with unharvested white clover, 282 stolon sections and 486 leaves were monitored in the pure stand and 65 stolons and 102 leaves in the mixture with meadow fescue. On plots with harvested white clover, 315 stolons and 478 leaves/petioles were monitored in the pure stand and 248 stolons and 428 leaves/petioles in the mixture. In order to reduce variation in leaf, stolon and root physical assessments, all observations were carried out by one person.

Root observations

Segments of visible nodal (diameter > 1 mm) and lateral (diameter < 1 mm) roots on the planted stolon segments and, later, nodal roots on newly emerged stolons were marked on the windowpane. Starting in July 2002, the physical condition of marked root segments was monitored weekly until mid October, and the monitoring was resumed the following April. In 2003, root condition was assessed every second week until mid October and then terminated in May 2004. On plots with grass–clover, roots of the two species were distinguished visually based on the occurrence of nodules on the white clover roots and the more cylindrical shape of meadow fescue roots. Dark brown or missing roots were assumed to be dead. On plots with unharvested white clover, 1089 root segments were monitored in the pure stand and 199 in the mixture with meadow fescue. On plots with harvested white clover, 1060 were monitored in the pure stand and 466 in the mixture.

Measurements of plant N and soil inorganic N

Harvested leaf material was dried (60 °C, 48 h) and N concentration (Kjeldahl-N) was measured. Plant root simulator probes (PRS™; Western Ag Innovations Inc., Saskatoon, Canada) were used to assess plant-available nitrate (NO₃-N) and ammonium (NH₄-N) in soil just after harvesting. Probes with anion and cation exchange membranes were buried in each plot. During a period of 1 week, the inserted probes were replaced by new ones every 48 h in the first year of the experiment and every 24 h in the second year. The PRSTM probes were extracted with 0.5 >N HCl (17.5 mL per probe), and the extracts were neutralized with NaOH. Nitrate and ammonium N in the extracts were measured colorimetrically by flow injection (Tecator, Sweden). Since ion uptake in the probes is not linear, the plant-available inorganic N is reported as the amount of nutrient adsorbed per unit area of adsorbing surface during the entire time of burial in the soil (Western Ag Innovations Inc., 2001).

Statistics

Statistical analyses were performed using the Minitab® Release 14.13, statistical software (State College, PA, USA). Descriptive statistics were used to characterize leaf, stolon and root longevity. Analysis of variance (ANOVA) was used to assess whether the treatments harvested or unharvested and pure stand or mixture, and whether time of plant part emergence (tagging date used as proxy variable) influenced the total lifespan. This was done according to a split-plot model with the treatment factors on main plots and tagging date representing subplots (i.e. repeated measurements of treatment effects on each main plot). Block was regarded as a random effect (replicate), and the effects of treatments and their interaction were tested against the plot-level error term (interactions with block), while the effects of tagging date and its interactions with the treatments were tested against the residual. As the combination of unharvested and mixture was missing in the first experimental year, the interaction of the two treatment factors could only be tested for the second year. Treatment effects on the winter survival of stolon sections, harvested N per plot and total N uptake in PRSTM™ probes were statistically tested by one-way ANOVA. Leaf, stolon and root lifespans were linearly regressed on daily mean air (2 m height) or soil temperature (10 cm depth) sums during the first month after emergence of the plant parts.

RESULTS

Lifespan of white clover plant organs

The longevity of leaves or petioles (from when leaflets were 90–95 % unfolded until they were dark brown or missing) ranged from 21 to 86 d regardless of treatment (Fig. 3) and temperature sum for the month after leaf emergence (results not shown). The leaf lifespan was not significantly longer in the first [65 ± 12 d (mean ± s.d.), n = 222] than in the second (53 ± 17 d, n = 76) growing season. Leaves emerging early in the growing season lived slightly longer than leaves appearing later on (P < 0.001; Fig. 4). For unharvested white clover plants, leaves along 61 and 59 % of the main stolon length had disappeared by late autumn in the first and second growing season, respectively (average for the pure stand and the grass–clover mixture; results not shown in the figures). For harvested plants, the corresponding values were 63 and 66 %, respectively. Of 60 leaves marked in October 2002, 76 % were dead or had disappeared by April 2003. In April 2004, the observed disappearance of leaves over the winter was similar.
The tagged sections of the main stolons lived from 111 to 677 d (Fig. 3) regardless of temperature sum for the month after stolon tagging (results not shown). Their mean longevity differed between unharvested and harvested plants (P < 0.001), but not between the pure stand and the grass–clover mixture. The lifespan of stolon sections varied with the time of emergence (P < 0.01; Fig. 4). The difference was particularly clear in the unharvested treatment, as can be seen from the percentages of initially tagged stolons assessed as dead (Fig. 5). The dead stolon sections underwent very slow decomposition and were visible for approx. 80 d after they were assessed as dead.

New roots developed mainly from May until July, while old roots died predominantly in the spring and in the autumn (Fig. 6). As the experiment proceeded and the white clover stand matured, the marked roots at the windowpane gradually turned brown and eventually disappeared. For root segments of unharvested plants marked during the first growing season (2002), 9 % had disappeared by the end of the first growing season, 13 % after the first winter, 76 % after the second growing season and 92 % by the end of the experiment (May 2004; results not shown in the figures). For the root segments marked during the second growing season, 26 % had disappeared by late autumn and 53 % by the end of the experiment. Altogether, 54 % of all root segments marked during the first and second growing seasons had decomposed by the end of the second growing season. At the end of the experiment, the corresponding figure was 74 %.

The roots lived between 27 and 621 d, and the average lifespan was unaffected by the harvesting treatments (Fig. 3). The root lifespan, however, depended on the time of their appearance (P < 0.001; Fig. 4). Root longevity also depended on the soil temperature sum in the month after root emergence (Fig. 7).

Plant N and soil inorganic N

In the first growing season, the total N yield of harvested white clover herbage was 5.4 g m⁻² in monoculture and 2.6 g m⁻² in the mixture with meadow fescue (results not shown in the figures). During the second summer, the clover yielded significantly more N, on average 17.6 g m⁻² in monoculture and 10.6 g m⁻² in the mixture (P < 0.05).
The total N yield of harvested meadow fescue herbage was 1.8 g m\(^{-2}\) in the first growing season and 4.4 g m\(^{-2}\) in the second growing season.

In 2002, the availability of soil NO\(_3\)-N and NH\(_4\)-N measured by PRSTM probes 48 h after harvest was similar for all treatments and differed little throughout the entire burial period (144 h; Fig. 8). In 2003, the availability of soil inorganic N was significantly larger on plots with unharvested white clover plants in pure stand already after 24 h than on plots that were harvested (\(P < 0.05\)). This difference remained throughout the burial period (Fig. 8). The availability of soil inorganic N tended to be larger in pure stand than in mixture with meadow fescue.

The three main white clover plant organs clearly had very different lifespans. The leaves were substantially more ephemeral than the stolons and roots (Fig. 3). Regardless of treatment, the mean leaf lifespan was 59 d, and leaves along approx. 60% of the main stolons senesced, died and decomposed during the summer. The leaves that emerged early in the growing season lived longer than leaves appearing later on, suggesting that leaf turnover was speeded up by increasing plant stand density (Thompson and Harper, 1988). Of the 40% still remaining alive in late autumn, about 70–80% died during the winter, and only traces of leaf debris were found in the spring. Repeated freezing and thawing incidents during the autumn and winter (Miller et al., 1994) and fungal damage under the snow cover (Sunde, 1996) probably accelerated leaf death. In the present experiment, deep snow cover restricted photosynthetic activity, suggesting that energy exhaustion due to respiration may also have

![Fig. 5](image-url) **Fig. 5.** Main stolon sections visually assessed as dead close to the parent fragment (emerged and tagged in June–July, 2002; see Materials and Methods for further explanation), in the middle of the main stolon (emerged and tagged in July–August, 2002) and close to the stolon apex (emerged and tagged in September–October, 2002) for unharvested and harvested white clover plants (mean of pure stand and mixture with meadow fescue). Vertical bars represent the standard error of the mean.

![Fig. 6](image-url) **Fig. 6.** White clover root emergence (positive values) and death (negative values) during 2003.

![Fig. 7](image-url) **Fig. 7.** Relationship between root lifespan (overall median) and soil temperature (0-1 m depth). Temperatures are calculated as sums of daily means for the month after root appearance.

**DISCUSSION**

The total N yield of harvested meadow fescue herbage was 1.8 g m\(^{-2}\) in the first growing season and 4.4 g m\(^{-2}\) in the second growing season.

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caused leaf death (Woledge et al., 1990). However, it seems likely that the inherently short leaf lifespan itself played an important role. Circumstantial support for this hypothesis comes from the results showing that leaf lifespan was independent of harvesting treatment (Fig. 3) and temperature after leaf emergence. Moreover, previous studies have shown that leaves die during the winter regardless of cultivars used under various conditions in temperate (Davies and Evans, 1982; Woledge et al., 1990) and northern temperate regions (Frankow-Lindberg and von Fircks, 1998; Sturite et al., 2006). This means that leaves may be a source of readily plant-available N (Mueller and Thorup-Kristensen, 2001) but also a pool of N potentially at risk to off-season loss (Korsaeth et al., 2002).

The white clover stolons were the most long-lived of the three plant organs investigated. Most stolons survived the winter, but sections near the terminal bud were less winter resistant than sections close to the parent fragment (Fig. 5). Eagles and Othman (1988) also found that winter damage resulted in loss of terminal buds in particular. In the present experiment, the main stolons remained on the soil surface or were partly aerial during the winter and, thus, were exposed to low temperatures before snow covered the white clover stand. This could have adversely affected winter survival (Hay, 1985; Frankow-Lindberg and von Fircks, 1988).

The white clover root integrity was largely maintained throughout the winter, and altogether root turnover was rather slow (mean longevity of 290 d, Fig. 3). Root death occurred mainly in the spring and towards the autumn (Fig. 6), and to a greater extent in the second than in the first growing season. Watson et al. (2000) found, in contrast to the present results, that white clover roots were very short lived in Italy (7–14 d) and in the UK (35-42 d). A possible explanation is methodological differences, but differences in temperature cannot be excluded as a cause. A high inverse correlation was found between the soil temperature sum during the month after root emergence and the recorded root longevity (Fig. 7), which agrees with the difference observed by Watson et al. (2000) between the results from the UK and Italy. Under controlled conditions, Forbes et al. (1997) similarly demonstrated a significant decrease in ryegrass (Lolium perenne) root longevity with increasing temperature. These results support the assumption that root turnover varies with the soil temperature, and they may largely explain the finding of a very long white clover root lifespan in the prevailing climate.

Harvesting negatively influenced stolon lifespan and led, in particular, to death of sections close to the parent fragment (Fig. 5). Deterioration of base sections during the summer of 2003 suggested that reallocation of resources to the apex region may have occurred, which is in agreement with a study carried out by Chapman and Robson (1992). In the present study, the death of stolon sections disrupted the physical continuity within the plants and broke them up into substantially smaller individuals towards the end of the 2003 growing season. A similar pattern has been observed in pastures under intensive sheep grazing (Brock and Hay, 1988) and when taprooted plants started nodal growth (Bonesmo and Bakken, 2005). Marriott and Smith (1992) found that 60 % of the aboveground stolon tissue present in the spring turned over during the growing season in grazed grass–clover swards in the UK. In the present experiment, it was found that 58 % of the main stolon length of harvested white clover plants had died, but had not completely decomposed, by late autumn. The results did not confirm the hypothesis that harvesting of the foliage increases root death (Evans, 1973) and results in a transient increase in soil N availability. Rather, the results from the PRSTM probe measurements in the second experimental year showed increased availability of inorganic N in soil with unharvested clover plants.

The N lost from dying or dead plant tissue may be subject to mineralization, microbial immobilization, incorporation into soil organic matter, loss to the environment, re-absorption by the plant it originated from or transfer to associated plants. The present data on loss of plant parts and data from Sturite et al. (2006) on N contents in leaves, stolons and roots of undisturbed white clover plants in 2002 on the Apelsvoll site enable a rough gross
estimate of undetected N input to the soil—plant system. This estimate includes re-allocation of N within the plants, re-absorption of mineralized clover N from the soil and N losses to the environment, but excludes rhizodeposition other than turnover of macroscopic roots as monitored in the present experiment. The N lost from plant tissue would then amount to 12 g N m$^{-2}$ during the first growing season and 51 g N m$^{-2}$ during the second. The total input of white clover-derived N to the soil—plant system due to turnover of plant parts during these two growing seasons (63 g N m$^{-2}$; winter loss excluded) corresponds to a 2.5-fold increase over the total N in harvestable shoots produced on the site during the same period. This is similar (2.2-fold increase) to what Høgh-Jensen and Schjoerring (2001) found in their study using the $^{15}$N technique. The present results support their finding that the N input from white clover to the soil is substantial and may exceed N in harvested shoots.

CONCLUSIONS

The leaves were the most dynamic part of white clover plants and substantially more ephemeral than stolons and roots. The inherently short leaf lifespan probably added to winter stress as an important cause of leaf death during the cold season, which may result in a substantial pool of N at risk to off-season losses. Stolons and roots lived substantially longer and were much more winter resistant than leaves.

The root lifespan was inversely correlated with the soil temperature but unaffected by harvesting. Harvesting led to an accelerated death of stolon sections close to the parent fragment and disrupted the physical continuity within the plants. However, harvesting did not influence leaf or root lifespan and did not result in any increased N availability in the soil.

From data on loss of plant parts during the two investigated growing seasons, it was calculated that the total gross N input to the soil—plant system corresponded to a 2.5-fold increase over the total N in harvestable shoots produced during the same period. This shows the considerable potential of white clover for the N status of clover-rich grassland.

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