Soil mineral nitrogen availability predicted by herbage yield and disease resistance in red clover (Trifolium pratense) cropping

Patrick McKenna · Nicola Cannon · John Conway

Abstract Nitrogen (N) is the most limiting nutrient in crop production. Legumes such as red clover can provide N through biofixation, but securing nitrogen in soil for subsequent crop production must also be considered. Variety selection and management in red clover cropping can influence soil mineral nitrogen (SMN) availability. A field trial to investigate this was conducted with six varieties, under one and two cut management, over 2 years. Dry matter (DM) and N yield, Sclerotinia resistance and SMN availability were assessed. Low DM and N yields (1.6–2.4 t DM ha^{-1} and 54–83 kg N ha^{-1}) in the first year of cultivation allowed * 40 kg N ha^{-1} to become available, but high DM and N yields (10.2–14.6 t DM ha^{-1} and 405–544 kg N ha^{-1}) allowed * 20 kg N ha^{-1} to become available. Wetter weather in 2015 caused significantly more SMN losses than 2016 (20 kg N ha^{-1} in 2015 and 5 kg N ha^{-1} in 2016). The varieties Amos, Maro and Milvus lost significantly more SMN in the winter period, which may have been caused by more severe infection of Sclerotinia (these varieties were 50–80% more severely infected other varieties). Varietal effect was non-significant for winter losses in 2016, where no significant varietal differences in Sclerotinia infection were observed. 1 cut made * 41 kg N ha^{-1} available in the growing season of 2015, whilst 2 cut made significantly less (37 kg N ha^{-1}). Cutting was non-significant in 2016 but 1 cut was less susceptible to losses in the winter period. Cutting in 2015 did not significantly affect herbage DM and N yields in the first or second cut of 2016.

Keywords Red clover Forage legume Soil fertility Nitrogen fixation Soil mineral nitrogen

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Introduction

Red clover (Trifolium pratense) is a perennial forage legume cultivated in temperate zones either in monoculture or combination with grasses. It is primarily associated with grassland livestock production but it can also be cultivated in arable rotations to build soil fertility (McKenna et al. 2018a), particularly in organic systems (Nykanen et al. 2000). Contributions to soil fertility from red clover (RC) cultivation are mainly viewed in the context of nitrogen (N) contributions, as the crop will fix up to 375 kg N ha^{-1} yr^{-1} under optimal conditions (Carlsson and Huss-Danell 2003), but other components such as increases in soil organic matter (Knebl et al. 2015) and soil aggregation (Miller and Dick 1995) also play a role. Many studies on biofixation in RC cropping have been undertaken (Dahlin and Stenberg 2010; Schipanski and Drinkwater 2011; Rasmussen et al. 2012), but these focus on N

Published online: 30 August 2018

availability in the aboveground herbage, which may be of little relevance for residual soil N.

The concentrations of soil mineral nitrogen (SMN—i.e. ammonium ? nitrate) can be used as a proxy to assess the contribution of legume crops to soil fertility (Peoples et al. 2017), although N released from senescing root systems will also be influential. This will only be consequential when the legume crop has been terminated and the subsequent crop planted. SMN availability can be used to assess contributions to soil N throughout the fertility-building phase, and this may be useful for predicting optimal management, particularly in perennial legume crops. As modern RC varieties can be reliably cultivated for 3 ? years (Abberton and Thomas 2011) and are managed in diverse ways (cut and remove, cut and mulch, green manure and grazing), understanding the dynamics of SMN availability in leys may assist farmers in optimizing the N elements of their contributions to subsequent soil fertility.

Modern varieties are also genotypically diverse, as RC is bee-pollinated and self-incompatible (Townsend and Taylor 1985). Three distinct classifications have emerged; early and late flowering, diploid/tetraploid and erect/prostrate. Early varieties flower 1–2 weeks before single varieties and provide more vigorous regrowth than late varieties in response to cutting (Madill and Skepasts 1981). Late varieties give most of their annual yield at the first cut and regrew with less vigour. Classification is more spectrum than dichotomy and some varieties may be described as 'intermediate', i.e. falling somewhere between early and late flowering.

Diploid varieties contain two sets of chromosomes (2n = 14), but tetraploid varieties contain 4 (4n = 28). These are bred artificially by plant breeders using chemicals such as colchicine or nitrous oxide (Evans 1954). Chromosome doubling in RC may improve agronomic performance, as polyploidy is associated with hardiness (Thompson and Lumaret 1992) and increased pest resistance (Nuismer and Thompson 2001). Increased disease resistance, particularly in clover rot (Sclerotinia sp.) is also reported for tetraploid RC by some authors (Vaverka et al. 2003; Vleugels 2013). Tetraploidy may also increase yields of DM and N (Frame 1976) due to the larger cells required by additional chromosomes (Anderson 1971).

RC naturally exhibits an erect growth habit (Bowley et al. 1984), but breeders have succeeded in creating prostrate varieties which produce stolons and can root at the nodes under optimal conditions (Rumball et al. 2003). These varieties were initially created in Australia and New Zealand by breeders seeking to increase persistence in response to grazing (Wrightson 2015). Prostrate varieties are not commonly grown in the northern hemisphere, but European breeders have released one prostrate variety (Pastor) for this market (Boller et al. 2012).

RC varietal diversity even extends to forage quality parameters such as polyphenol oxidase (Eickler et al. 2011) and isoflavone (Papadopoulos et al. 2006) content, but this study focused exclusively on phenology, ploidy and growth habit. The objective was to investigate the effect of RC variety and cutting management on the availability of SMN over two growing seasons (March 2015–February 2017). As availability may be influenced by aboveground dry matter (DM) yield, N yield and disease resistance, this was also documented.

Materials and methods

The field experiment was established on a clay, shallow, lime-rich soil of the Sherborne series (Avery et al. 1980). This series is characterized by a calcareous topsoil of 250–350 mm depth over a thin subsoil that quickly passes to limestone. It is commonly referred to as 'Cotswold Brash'. A chemical and physical characterization of the site soil was undertaken before the trial began, the results of which are given in Table 1.

Meteorological conditions for 2015 and 2016 are given in Figs. 1 and 2. Data for 2015 and 2016 is taken from the weather stations at the Royal Agricultural University and long term average data (1980–2010) is taken from the Met Office data for Cirencester (2010). January 2017 received 69 mm of rainfall and a mean temperature of 13.8 C.

Experimental design

Experimental design was completely randomized block with four replications. Each block measured 6 9 36 m and was composed of 24 plots, each 9 m^2 (3 9 3 m). Each plot was assigned two treatments:

Table 1 Soil parameters at field trial initiation (April 2015)

Parameter	Data	Methodology
% Moisture (at sowing)	27.1	
pH	7.1	pH in water
Bulk density (g cm^{-3})	1.1	
Total carbon %	3.8	Calculated via analysis with Elementar Cube
Inorganic carbon %	1.8	Calculated using reaction with HCl in calcimeter
Organic carbon %	2	Calculated as the difference between total carbon and inorganic carbon
Organic matter %	3.4	Calculated by multiplying organic carbon by 1.72 (Grewal et al 1991)
Soil mineral nitrogen (kg ha^{-1})	12.7	Calculated via extraction in potassium sulphate and analysis using FIAstar
Phosphorus (mg L^{-1})	11.1	Olsen P
Potassium (mg L^{-1})	234.1	Calculated via extraction in ammonium nitrate (Faithful 2002)

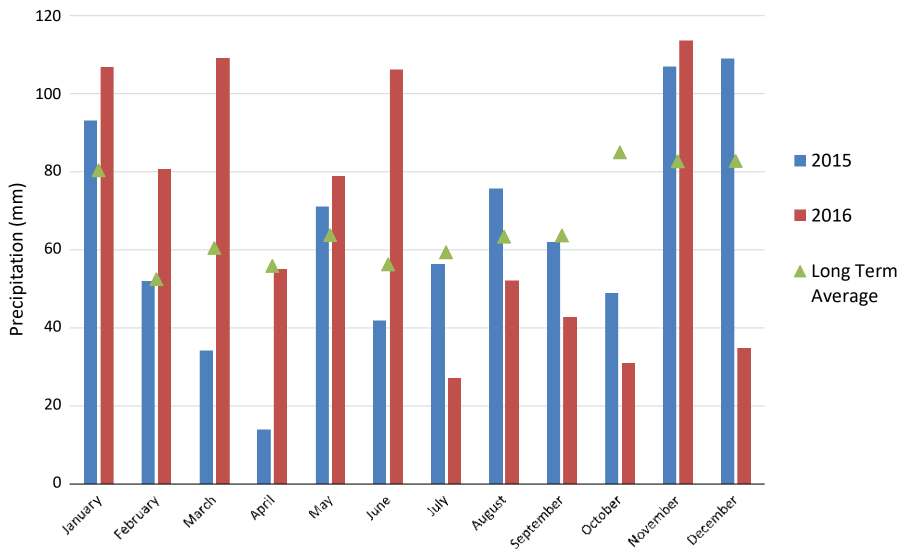

Fig. 1 Total precipitation (mm) in year 2015 and 2016 with 1980–2010 long term average

RC variety (Amos, Astred, Claret, Maro, Milvus, Ruby) and cutting management (1 and 2 cuts).

Materials

Six varieties of RC were selected to be evaluated. Attempt was made to select varieties representative of all available categories, but as large treatment numbers would require much replication and reduce statistical power (Clewer and Scarisbrick 2001), a limit was set at six. As the study took place in Britain, a balance was struck between fully representing all categories and selecting varieties likely to be used by British farmers. For example, as early varieties are more commonly cultivated in Britain, more early varieties were selected. Selected varieties and their description are given in Table 2.

Cultivation

The field selected for trial had previously been a timothy (Phleum pratense) ley. The site was sprayed with glyphosate (N-(phosphonomethyl)glycine)) on April 24th 2015 and then harrowed on April 27th.

The trial was sown on April 29th. Seeds were sown by hand at the advised rate of 15 kg ha^{-1} (Frame et al. 1998). The field trial soil was known to contain Rhizobium leguminosarum biovar trifolii and therefore no prior inoculation was required. Following sowing the whole site was rolled with a mini-roller to ensure good seed/soil contact for optimal germination.

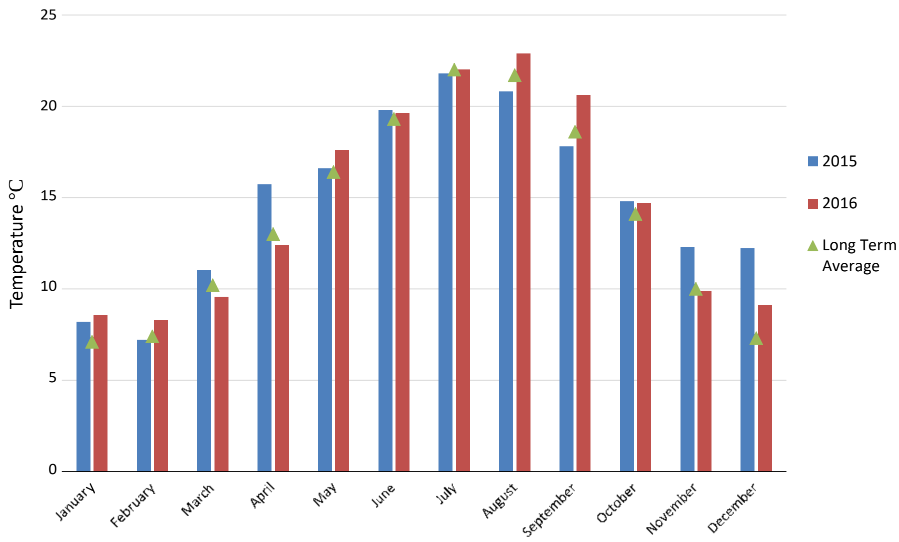

Fig. 2 Mean temperature (C) in year 2015 and 2016 with 1980–2010 long term average

Table 2 Description of red clover varieties selected for evaluation

Variety	Flowering	Ploidy	Growth habit	Seed merchant
AberClaret	Early	Diploid (2n)	Erect	IBERS (UK)
AberRuby	Early	Diploid (2n)	Erect	IBERS (UK)
Astred	Early	Diploid (2n)	Prostrate	PGG Wrightson (Australia)
Milvus	Early	Diploid (2n)	Erect	DSV (Germany)
Amos	Late	Tetraploid (4n)	Erect	DLF Trifolium (Demark)
Maro	Late	Tetraploid (4n)	Erect	DLF Trifolium (Denmark)

A dry period followed immediately afterwards and germination did not occur until May 6th (following a spell of rain). This delay allowed weeds to establish but the plots were left to establish without manual weeding.

Treatments of one and two cuts were applied to all varieties (i.e. all plots were cut once and then half were cut twice). Cutting was done by hand, with care taken to avoid damaging the crowns. All cuttings were removed, bagged and dried at 105 C for 24 h for DM and N content. N content was determined using an Elementar Cube. Dry plant material was fine-ground to 2 mm particle size and 25 mg samples were weighed into foil boats. 25 mg tungsten oxide (W_2O_3) was added as a combustion agent. The first cut began when the earliest flowering varieties between early and half bloom, the recommended cutting time for RC (Hall and Eckert 1992). The second cut was then applied at the same growth stage. Varieties were cut one by one in the order in which they flowered (Ruby–Milvus–Claret–Astred–Maro–Amos). Cutting began on July 30th and October 12th in 2015, and June 14th and August 10th in 2016 for first and second cut respectively. These and SMN assessment dates are included in the field operations diary (Table 3).

SMN availability

Soil mineral nitrogen (SMN) was determined using the potassium sulphate (K_2SO_4) extraction method (Faithfull 2002). Soil cores of 0.1 m diameter were taken from a 0–0.3 m depth. These samples were then immediately taken to the lab for extraction, to prevent any subsequent mineralization creating inaccurate measurements. Extract mineral N was calculated as the sum of the nitrate and ammonium concentrations determined with a Flow Injection Analyzer (FIAStar 5000) and converted to kg N ha^{-1} using the formulae of Unkovich et al. (2008).

Time constraints in 2015 prevented SMN assessments at the times of cutting (June and October). An

Table 3 Diary of field operations 2015–2017

2015		2016		2017	
Date	Field operation	Date	Field operation	Date	Field operation
April 24–29	Site preparation and soil characterization	February 4	SMN assessment	February 5	SMN assessment
July 30–August 10	First cut and harvest	June 14–23	First cut and harvest		
October 12–22	Second cut and harvest	June 15	SMN assessment		
November 5	SMN assessment	August 10–19	Second cut and harvest		
		August 21	SMN assessment		

SMN soil mineral nitrogen (ammonium and nitrate kg ha^{-1} extracted from 0.3 by 0.1 m soil cores in potassium sulphate)

assessment was therefore undertaken on November 5th 2015. In 2016 assessments took place on February 4th, June 15th and August 21st. A final assessment was undertaken on February 5th 2017.

Disease scoring and management

Sclerotinia sp. (clover rot) was observed on October 25th 2015 and scored on November 1st according to Dixon and Doodson (1974). Ten individual plants were randomly selected from each plot and assigned a value of 0–3 according to an illustrated key (no symptoms 0, slight 1, moderate 2 and severe 3). A disease index was then calculated according to the formula:

$$\text{Disease Index} = 10(x + 2y + 3z)$$

where x, y and z are the numbers of plants rated 1, 2 and 3.

A hydantoin fungicide (Iprodione—3-3,5-dichlorophenyl) was applied at 2 kg ha^{-1} using a knapsack sprayer on November 2nd. The late infection and cutting date precluded assessment of the effect of cutting on disease susceptibility, as minimal regrowth had occurred following the second cut. The disease recurred on August 25th 2016 but no fungicide was applied as no further growth was required for research in 2017. The disease was scored on November 1st and the effect of cutting management was assessed as sufficient regrowth had taken place in the plots which had been cut twice.

Statistical analysis

Data was analysed using analysis of variance (ANOVA) with the completely randomized block design. The repeated measurements function was used to incorporate the temporal element in the availability of SMN. The standard error of the difference between the means (SED) was used to test differences between treatments. All statistical analyses were carried out using GENSTAT statistical software (Genstat 19th Edition 2018, VSNI).

Results

Dry matter and nitrogen yields 2015

The intermediate and late varieties Maro and Amos yielded significantly more DM than the early varieties at the first cut (Table 4). Milvus yielded significantly lower DM than the other early varieties (Table 4). Establishment was slow due to dry conditions between April 29th and May 4th. Weed populations established in all plots and weed DM was assessed. Maro and Amos yielded significantly more N than the early varieties and Astred yielded the lowest N (Table 4). Weeds composed $< 5\%$ of overall biomass at the second cut. Weed DM was then considered negligible and not recorded. The early diploids yielded significantly more DM and N than the late tetraploid Amos at the second cut (Table 4). However, the intermediate variety Maro maintained good growth and yielded the same DM and N as the early varieties (Table 4). When both cuts were summed Amos yielded significantly less DM and N than all other varieties and no significant differences were observed for all other varieties (Table 4).

Table 4 Dry matter and nitrogen yields of red clover and weeds in 2015 and 2016

	2015							2016					
	First cut			Second cut		Total		First cut		Second cut		Total	
	Clover (DM t ha^{-1})	Weed (DM t ha^{-1})	Clover (N kg ha^{-1})	Clover (DM t ha^{-1})	Clover (N kg ha^{-1})	Total (DM t ha^{-1})	Total (N kg ha^{-1})	Clover (DM t ha^{-1})	Clover (N kg ha^{-1})	Clover (DM t ha^{-1})	Clover (N kg ha^{-1})	Clover (DM t ha^{-1})	Clover (N kg ha^{-1})
Amos	0.75a	0.64c	24.4ab	0.82c	30.0c	1.58b	54.4b	8.70b	330.0a	4.06b	159.7b	12.24ab	472.0ab
Astred	0.56bc	0.65bc	15.8c	1.57a	67.0a	2.11a	82.8a	6.07c	236.7b	4.51b	183.0b	10.17b	403.3b
Claret	0.61b	0.68abc	19.3bc	1.64a	61.9ab	2.20a	81.1a	6.28c	217.5bc	4.93ab	184.0b	11.61b	399.5b
Maro	0.85a	0.79a	28.1a	1.51ab	56.6b	2.39a	83.2a	9.74a	351.3a	4.32b	177.1b	14.66a	544.1a
Milvus	0.49c	0.76ab	19.3bc	1.60a	61.8ab	2.17a	80.9a	6.40c	232.5b	5.22ab	179.5b	11.59b	405.5b
Ruby	0.56bc	0.64c	19.2bc	1.35b	55.9b	1.95ab	75.6a	5.00d	191.6c	6.37a	231.9a	11.37b	414.7b
Df	87	87	87	39	39	39	39	87	87	39	39	39	39
SED	0.056	0.056	3.16	0.11	4.69	0.15	6.27	0.48	17.22	0.49	19.81	0.74	27.12
p	\ 0.001	\ 0.001	\ 0.001	\ 0.001	\ 0.001	\ 0.001	\ 0.001	\ 0.001	\ 0.001	\ 0.001	0.017	\ 0.001	\ 0.001

Values followed by the same letter do not significantly differ at cited p value

SED standard error of the difference, Df degrees of freedom, DM t ha^{-1} dry matter tonne per hectare, N kg ha^{-1} nitrogen kg per hectare

Dry matter and nitrogen yields 2016

Significant differences for both DM and N yields were observed at both cuts. The later varieties yielded significantly more than the early varieties at the first cut and the intermediate flowering Maro yielded significantly more than the late variety Amos (Table 4). Ruby yielded significantly less DM than the other early varieties, for which no differences were observed (Table 4). A similar pattern was observed for N at the first cut, although no differences were observed between Amos and Maro (Table 4). Ruby gave the highest yield at the second cut, but this was equal to that of Milvus and Claret. Amos, Astred and Maro yielded the same as Milvus and Claret, but lower than Ruby (Table 4). Ruby yielded significantly more N at the second cut than all other varieties, for which no differences were observed (Table 4).

Maro and Amos yielded significantly more DM and N than all other varieties when both cuts were summed and no significant differences were observed between Amos and Maro. A range of 399–544 kg N ha^{-1} was reported and the significant differences between varieties were the same as that of DM yield (Table 4).

Disease incidence and resistance 2015 and 2016

Ruby and Astred were found to be significantly more resistant to clover rot than all other varieties (p \ 0.001) in 2015 (Table 5). No significant differences were observed for Claret, Maro and Milvus, whilst Amos was the most susceptible (Table 5). No significant varietal effects were observed in 2016 but 1 cut was more susceptible than 2 cut (p \ 0.001, Table 5).

SMN availability 2015 and 2016

All varieties significantly increased SMN availability in the growing season of 2015 (12.5 to * 40 kg N ha^{-1}), but no significant differences were observed among varieties (Fig. 3). Astred, Claret and Ruby were significantly less susceptible to SMN loss than Amos, Maro and Milvus over the wet winter of 2015 (Fig. 3). Variety did not significantly affect SMN availability in the growing season of 2016 and also did not affect susceptibility to losses in the winter period of 2016 (Fig. 3).

Table 5 Disease index of clover rot 2015 and 2016

Variety	Disease index 2015	Disease index 2016
Amos	258.8c	177.5ns
Astred	136.2a	178.8ns
Claret	225.0b	167.5ns
Maro	213.8b	168.8ns
Milvus	245.0bc	175.0ns
Ruby	136.2a	202.5ns
SED (33 Df)	9.44	16.81
p	\ 0.001	ns
Cut 1		229.2a
Cut 2		127.5b
SED (33 Df)		9.71
p		\ 0.001
Higher score indicates higher infection		

Values followed by the same letter do not significantly differ at cited p value

SED standard error of the difference, Df degrees of freedom

1 cut significantly increased SMN availability in the growing season of 2015, but had no effect on susceptibility to winter losses (Fig. 4). Cutting then had minimal effect on SMN availability throughout the growing season of 2016, but 2 cut was more susceptible to losses in the winter period of 2016 (Fig. 4).

Astred and Ruby significantly raised SMN availability when 1 cut was applied in 2015, but Maro raised SMN availability when 2 cut were applied (Fig. 5a, e). Ruby, Milvus and Claret were less susceptible to winter losses when 1 cut was applied in 2015, no significant interaction was observed for Astred and Maro, and Amos was more susceptible to losses when 2 cuts were applied (Fig. 5a, b, d). Some significant interactions were observed throughout the growing season of 2016, although the effects were mixed and overall SMN availability did not change as much as it did in 2015. Most varieties incurred losses in the 2 cut treatment over the winter period of 2016, but in Amos these losses occurred in the 1 cut treatment (Fig. 5a–e).

Discussion

DM and N yields

DM yields are important for livestock farmers and N yields are often used as a proxy to estimate nitrogen

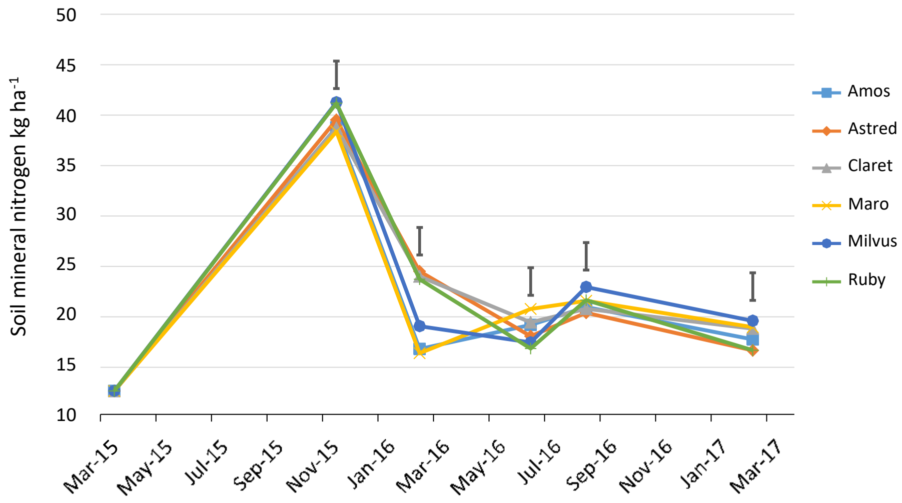

Fig. 3 Effect of red clover variety on soil mineral nitrogen availability. Error bars indicate standard error of difference between means

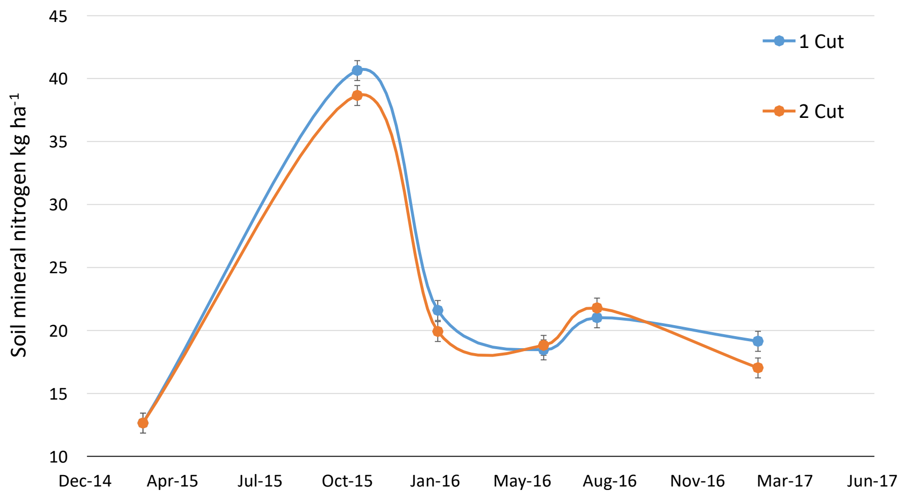

Fig. 4 Effect of cut on soil mineral nitrogen availability. Error bars indicate standard error of difference between means

fixation rates (Carlsson and Huss-Danell 2003). Tetraploid RC is cited as yielding more DM and N in the aboveground biomass than diploid (Frame 1976; Vleugels 2013; Amdahl et al. 2016). The tetraploid varieties trialled here (Amos and Maro) yielded higher DM and N than the diploids at the first cut in both years of cultivation, although the primary cause of this was more likely the later flowering date than ploidy, as later flowering varieties are expected to yield the bulk of their yield at the first cut. Regrowth following cutting was limited in Amos in 2015 due to the later cutting date, but in 2016 it gave yields comparable to the early diploids. DM yields for all varieties were curtailed in 2015, likely due to weed pressure following establishment, but comparable to some reported ranges in 2016 (Marley et al. 2013; Elsaesser et al. 2016; Nagibin et al. 2016 all reported DM yields of 10–15 t DM ha^{-1}). The only prostrate variety, Astred was bred specifically for grazing management, and therefore may not yield as much as erect varieties (Rumball et al. 2003) and but was shown here to yield DM and N that was comparable to the other erect varieties in both years.

Higher DM yields for RC have however been reported, particularly when combined with grasses (Marshall et al. 2012, 2017). RC/grass combinations may then yield more DM than monocultures and the faster establishment of grasses may offset weed establishment in the first year of cultivation. Weed suppression 2015 was also notable, and may have been caused by the exudation of allelopathic chemicals in response to cutting (Ohno and Doolan 2001), although

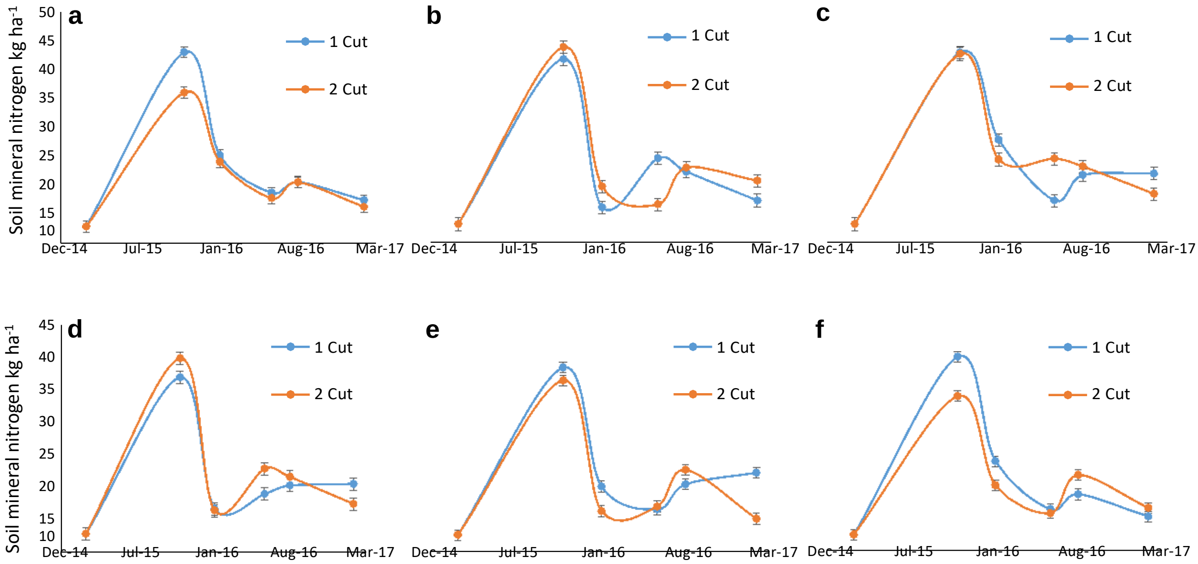

Fig. 5 Effect of interaction between time and cut on soil mineral nitrogen availability in a Astred, b Amos, c Claret, d Maro, e Milvus and f Ruby. Error bars indicate standard error of the difference between the means

weed growth may also have been curtailed by the cutting treatment. This may be a promising area for future research in RC breeding and agronomy, but it is omitted from this study as no significant varietal or management effects were observed.

Herbage yields of 54–83 kg N ha^{-1} in 2015 were comparable to some reported ranges (Heichel et al. 1985; Sparrow et al. 1995; Huss-Danell et al. 2007), but total yields of 405–544 kg N ha^{-1} in 2016 are outside the reported range of most studies—a comprehensive review of N yields in RC reported a highest value of 375 kg N ha^{-1} (Carlsson and Huss-Danell 2003). These studies however focus on fixed N (% N derived from atmosphere—% Ndfa), not total N yields. % Ndfa was likely high in this study, given how low initial SMN availability was in 2015 (12.5 kg N ha^{-1}—Table 1), and throughout the growing season of 2016. Total N yield in RC is also influenced by the yield potential of the variety (Thilakarathna et al. 2016a) and in this study tetraploidy may have increased N yields in Maro in 2016, and facilitated Amos in yielding the same N as the early diploids at the second cut, despite expectations of a reduced second cut because of its phenology. Increased N yields may be the most significant effect of tetraploidy in RC, as tetraploids not only produce significantly larger cells than diploids (Anderson 1971), but also up to 40% more leaf area (Thilakarathna et al. 2018). This can increase yield potential and provide additional C from photosynthesis to nodules, thus increasing fixation and N uptake.

Disease resistance

Some studies predict higher clover rot resistance in tetraploid RC (Vaverka et al. 2003; Vleugels 2013). Here the diploid varieties Astred and Ruby were more resistant than the tetraploids in 2015, but no significant varietal effects were observed in 2016. Clover rot is considered a serious disease by most authors and significant yield reductions are expected in the subsequent year of cultivation, as the disease can damage taproots and curtail regrowth the following year (Vleugels 2013; Mikaliuniene et al. 2015). This was not observed in this study, as yields were much higher in 2016 than 2015. The fungicide application in late 2015 may have controlled the disease to an extent which facilitated good growth in 2016. Clover rot is common but fungicides are not commonly applied, likely because they are thought to be ineffective at terminating sclerotia and preventing the return of the disease the following year. What would have happened without the fungicide application in this study remains unclear, but DM and N yields were high despite disease recurrence.

Two cuts significantly reduced clover rot incidence in 2016. Regrowth following multiple cuts in RC will generally require translocation of N from roots to shoots (Volenec et al. 1996), at least in the immediate time afterwards, and cutting can also cause nodule senescence and limit the available supply of fixed N (Jarvis et al. 1996). Defoliation stress can reduce root N content (Thilakarathna et al. 2016b), which can also reduce fungal infection, as fungi will prefer to attack N rich sources. This is also consequential for SMN availability, as N may also be exuded from the root system following cutting and may contribute to N losses in wet conditions. Prostrate varieties are also cited as growing a root system more reliant on adventitious roots than a single deep taproot, to facilitate its capacity to grow stoloniferously (Vleugels 2013) and this reduced N source in its roots may explain its increased resistance to clover rot in 2016.

SMN availability

SMN availability was high in 2015 when aboveground N yields were low, and low in 2016 when aboveground N yields were high. The reported range was higher than that of Thilakarathna et al. (2016a) in 2015, but comparable in 2015. Both years were significantly lower than Kayser et al. (2010), who reported availability of up to 60 kg N ha^{-1}. Herbage N yields were lower in 2015 than 2016 (Table 4), but were likely facilitated by favourable nodulation rates, as low SMN availability is associated with increased nodulation (Thilakrathna et al. 2012). Varietal effects were minimal (Fig. 3) but Astred, Ruby and Claret scored significantly higher for SMN in the February 2016 assessment, implying these varieties were less susceptible to winter losses.

Significant reduction of SMN availability occurred in the winter of 2015–2016, likely exacerbated by high rainfall in December (Fig. 1). Cutting was non-significant for SMN availability in winter losses in 2015, but 1 cut lost significantly less in the winter of 2016 (Fig. 4). Losses were also less significant in 2016 than in 2015, but there was lower SMN available to be lost in this period. Changes in SMN in the growing period of 2016 were minimal, although some significant differences were observed in the interaction between variety and cut (e.g. the 1 cut treatment in Claret significantly reduced SMN between February and June—Fig. 5c, and the 2 cut treatment in Milvus significantly increased SMN between February and August—Fig. 5e). The increased N demand of the aboveground herbage in 2016 is likely to have limited the availability of SMN in this period, but wetter conditions, particularly in June 2016 (Fig. 1) may also have contributed by leaching N from the system prior to the SMN assessment. Leaching susceptibility is of particular concern in RC monocultures where companion grasses are not available to take up SMN (Moyo et al. 2015).

Optimal management in SMN availability in RC

RC cultivation and management across cropping systems is diverse. Diversity among varieties is also predicted, and the results of this study indicate these predictions are well-founded. Adoption of RC into crop rotations can build soil fertility and reduce reliance on mineral fertilizers, and research into the fertility-building capacity of modern varieties under different management may further optimize its use and increase adoption rates.

Appropriate disease management can optimize the soil fertility contributions of RC cropping. Ruby and Astred were clearly more resistant to clover rot than all other varieties in 2015 ($p \setminus 0.001$) but given how this assessment only took place on the '1 cut' plots, drawing conclusions about the interaction between this infection, cutting management and SMN availability is difficult. However studies with fewer variables tend to cite tetraploids as more resistant to clover rot (Frame 1976; Vaverka et al. 2003; Vleugels 2013) and fungicide applications as being ineffective at controlling clover rot once it occurs (Ohberg and Bang 2010).

Varietal resistance and fungicides may maintain high yields, but the interaction between aboveground yields and SMN availability indicate high yields may not always be desirable. High aboveground N yields can reduce SMN availability, but this can change when returned to the soil as a green manure following termination. This dynamic between aboveground and belowground N has been described in other studies, most prominently in grain legumes with high N demands (Giller 2001). Soybeans even have the potential to leave an SMN deficit following cultivation (Dukic et al. 2014). This phenomenon is generally thought not to occur in forage legume cultivation (Anglade et al. 2015), as the N demand of the much smaller seed will be much lower, however the high-N demand of the herbage in 2016 in this study must have depleted SMN to a degree, as SMN availability was high when aboveground N yields were low, and vice versa.

This may be circumvented by returning high yields of aboveground herbage to soil as green manure or mulch. Mulching the first cut when N yields are high may however result in the subsequent regrowth using the senescing mulch as its N source, a process known as N 'recycling' (Dahlin et al. 2011). Recycling is undesirable in fertility-building, at least throughout the growing season, in which the majority of annual biofixation takes place (Ledgard and Steele 1992), because it limits biofixation and will prevent the senescing mulch from entering the soil as SMN.

This seems intuitive, as all cuttings were removed following cutting and therefore the 1 cut treatment both returned more N to the soil (when senescing plant tissues mineralized) and also received more soil cover (from the physical presence of the uncut and unremoved biomass on the topsoil). This soil cover may also prevent leaching of other soil nutrients and contribute to the soil organic matter pool, a major benefit of using RC in conservation tillage systems (McKenna et al. 2018b). If cut and remove management is to be applied then it may be preferable to cut only once. If cut and mulch is to be practiced then a second cut may be preferable.

Conclusions

High aboveground N yields, although desirable for forage production, may contribute little to SMN availability. SMN availability was most enhanced when aboveground biomass was cut once, and when disease resistance was highest. The tetraploid Maro consistently produced the highest yields of DM and N, but was not as resistant to clover rot as the diploids Ruby and Astred. Cutting and removing biomass can reduce clover rot infection.

Acknowledgement We are grateful for the seed and support provided by Ian Wilkinson and David Lloyd at the beginning of this project.

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