The use of red clover (*Trifolium pratense*) in soil fertility-building: A Review

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A R T I C L E I N F O

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A B S T R A C T

Red clover cultivation made significant contributions to soil fertility prior to the introduction of mineral nitrogen fertilizers. Its modern usage lies primarily in forage production, but reintegration into arable systems can en- hance sustainability and preserve environmental integrity. Here we review red clovers nitrogen (N) contribution to subsequent crops, its capacity to fix N, and how this N is transferred to subsequent crops. The senescence of the root system following cultivation also contributes to soil organic matter, providing a suite of ecosystem services which are also reviewed. Potential contributions to allelopathic weed control and how this may be utilized to improve weed control is also discussed. Red clover varieties are diverse and can be split into cate- gories of early/late flowering, erect/prostrate and diploid/tetraploid. This use of this diversity to different ends and purposes in fertility-building and the role of plant breeding in optimizing use of genetic resources is re- viewed. Management strategies are also diverse; red clover can be grown in monoculture or with companion grasses, it can be harvested for forage or green manured (which can include or omit herbicides) and the con- sequence of this for soil fertility is discussed. High protein forage production is also a key benefit of red clover cultivation and the economic incentive this may provide to farmers is also reviewed.

1. Introduction

Red clover (RC) is a forage legume cultivated in the temperate world, noted for its high-protein feed (Marshall et al., 2017) and high rate of biological nitrogen fixation (BNF) (Dhamala et al., 2017). This review will focus on the use of RC in soil fertility-building, the contribution it can make to sustainable intensification and the reduction of agriculture's environmental impact. RC was historically cultivated in rotations with other crops to maintain yields, but the advent of mineral fertilizers in the 20th century has displaced much of this use. It remains used for this purpose in many organic systems (Nykanen et al., 2000), but modern usage now lies mostly in grass/clover leys for forage (Abberton and Marshall, 2005). The traditional role of RC as a fertility- building crop remains, however, underutilized and this review will focus on this use.

2. Historical perspective

Wild RC is thought to have originated in South East Eurasia and was first cultivated by farmers in Europe as early as the third century (Taylor and Quesenberry, 1996). Its use in fertility-building and forage production was ubiquitous by the 16th century (Mousset-Déclas, 1995), a dual role cited as having more impact than the introduction of the potato (Fergus and Hollowell, 1960). Replacement of fallowing with RC cultivation increased productivity (Rham, 1860), as did the Norfolk 4 rotation of wheat-turnip-barley-clover (Knox et al., 2011).

The capacity of RC to increase productivity was also recognized by Thomas Jefferson, who wrote in a letter to a friend;

> '*Horizontal and deep ploughing, with the use of plaister and clover, which are but beginning to be used here will, as we believe, restore this part of our country to its original fertility*' (Jefferson, 1817)

Of course, Jefferson wrote this a century before the Dust Bowl and was unaware of the effect 'horizontal and deep ploughing' would go on to have in parts of America (Baveye et al., 2011), but he was ahead of his time in understanding the importance of leguminous rotations in the maintenance of soil fertility. RC cultivation is now almost global. Recommended growing conditions are summarized in Table 1, along with its reported distribution and bioactive compounds.

3. Prospects for red clover in soil fertility-building

Concerns over the potential environmental impact of mineral fertilizers have revived interest in the use of forage legumes to build soil fertility in rotations with cereal crops (Taylor, 2008a), but the high N demand of cereals is a challenge in lower-input systems (Gooding and Davies, 1997). Timely residual N release (i.e. during the spring growth season) is also essential for cereal production. Doel (2013) investigated

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Table 1
Summary of RC distribution, growth conditions and bioactive compounds.

Global Distribution	North America, Europe, Northern China/Japan, Southern Latin America/Australasia		(Frame et al., 1998)
	Survival Range	Optimal Range	
Soil pH	5.0–8.5	6.0–7.6	(Rice et al., 1977)
Temperature	7–40 °C	20–25 °C	(Frame et al., 1998)
Annual Precipitation	350 mm Upwards	550 mm Upwards	(Frame et al., 1998)
Soil Drainage	Poorly-Well Drained	Well Drained	(Wyngaarden et al., 2015)
Soil Salinity	0–1.5 dS/m	0–0.75 dS/m	(Rogers, 2008)
Bioactive Compounds	Polyphenol Oxidase		(Lushcer et al., 2014)
	Isoflavonoids (Phytoestrogen)		(Boue et al., 2003)

the impact of various fertility-building plants on the yield of subsequent winter and spring wheat in the UK, and reported significantly higher yields following RC cultivation. Moyo et al. (2015a,b) investigated how management practices and companion grasses can significantly affect cereal yields in the same site. These studies are summarized in Figs. 1 and 2.

The results of Figs. 1 and 2 are taken from studies over two years in Gloucestershire, UK. The soil type was calcareous clay loam over Oolitic limestone, commonly referred to as Cotswold Brash (Avery et al., 1980). Wheat was planted following preceding cropping treatments on October 20th and February 19th for Fig. 1, and on 14th October and 14th March for Fig. 2. All crops were sown by hand broadcast, apart from barley, which was drilled. All preceding cropping treatments were for one year and no fertilizers were applied. The data summarized in Fig. 1 (Doel, 2013) indicates RC significantly impacts on subsequent crop production in short time periods. The data summarized in Fig. 2 (Moyo et al., 2015a,b) indicates management and companion grass treatments had significant effects on cereal production. Figs. 1 and 2 demonstrate the fertilizer value of RC cultivation by translating it into grain yield of subsequent crops, which are comparable to conventional mangement in some cases.

4. Nitrogen for contemporary agriculture

Quantification of N inputs is beset by uncertainties and the only reliably accurate statistics for agricultural inputs are for mineral fertilizers; however, some notable estimates have been calculated by Smil (1999b) and Galloway et al. (2004). The European Nitrogen Assessment Project has also made estimates of N cycling in European agriculture using modelling data (Leip, 2011).

Knowledge of the various N sources in global agriculture helps to contextualize the contribution of legumes, but describing the flow of N in agriculture is difficult. Crop residues have many uses (various fuels, fodders and fibers) and no country keeps comprehensive statistics of their uses, making it difficult to assess their contribution to soil N. It is also difficult to calculate the N content of manure from stock under different systems of production, no less to determine what percentage is returned to the soil after manuring. Furthermore, as this review outlines, the numerous methods of measuring fixation in the nodules of legumes have produced varying accounts of the contribution of fixation to the overall N economy. Examples of estimated global and regional N inputs are given in Table 2.

These estimations include both natural and anthropogenic inputs. Understanding how sustainable sources of N derived from BNF can be optimized requires an understanding of the biogeochemical cycling of N in systems using legumes. This can be split into three components; biological fixation of atmospheric nitrogen by Rhizobia bacteria living in the root nodules, the subsequent return of organic N to the soil and the uptake of this by subsequent crops (Cuttle et al., 2003). BNF can be limited or enhanced by soil N status; establishment/persistence, geno- typic variation and stresses (Cherr et al., 2006), and mineralization rates are influenced by the C/N ratio of crop residues, management strategies, weather and soil microbe activity (Sarrantonio and Scott, 1998). This means that a variety of agronomic factors must be con- sidered to optimize N contributions from RC cultivation.

5. Biological nitrogen fixation

A number of studies documenting the volume of nitrogen fixed by RC under varying management strategies have been conducted. Tables

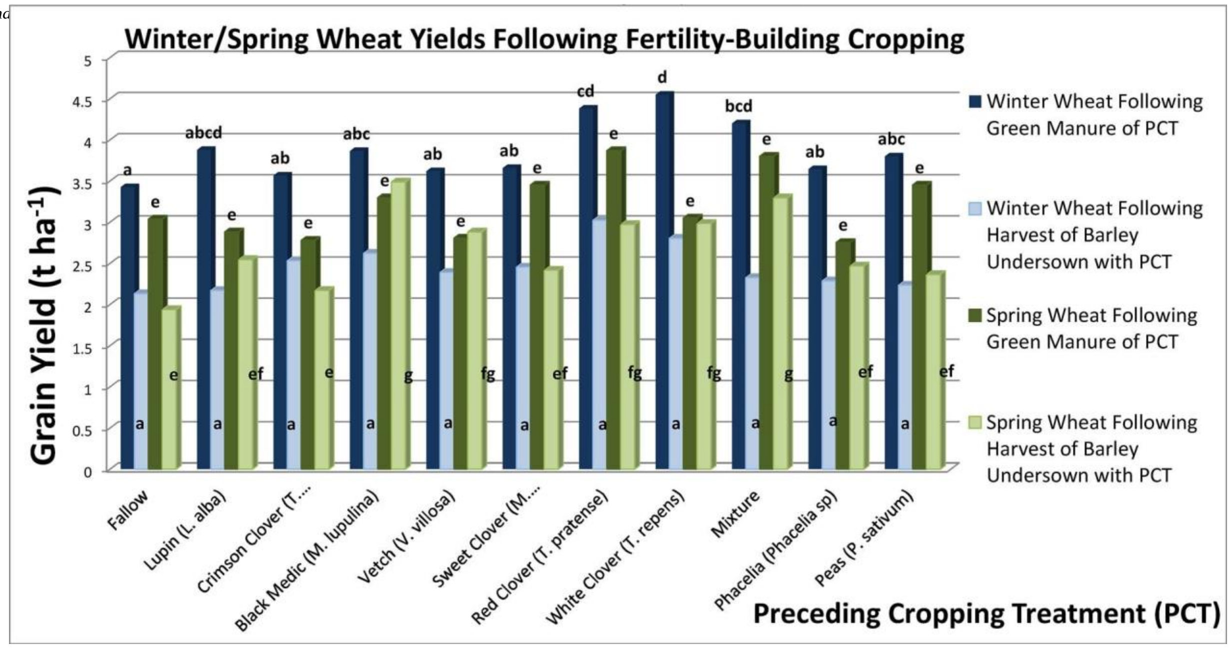

Fig. 1. Impact of various fertility-building crops on winter/spring wheat yields over 6 and 12 months with differing management strategies (Doel 2013).
Fallow results indicated control plots with natural regeneration and 'mixture' indicated 40% RC, 30% sweet clover, 15% lupin and 15% black medic. Bars with the same letters are not significantly different ($P < 0.05$).

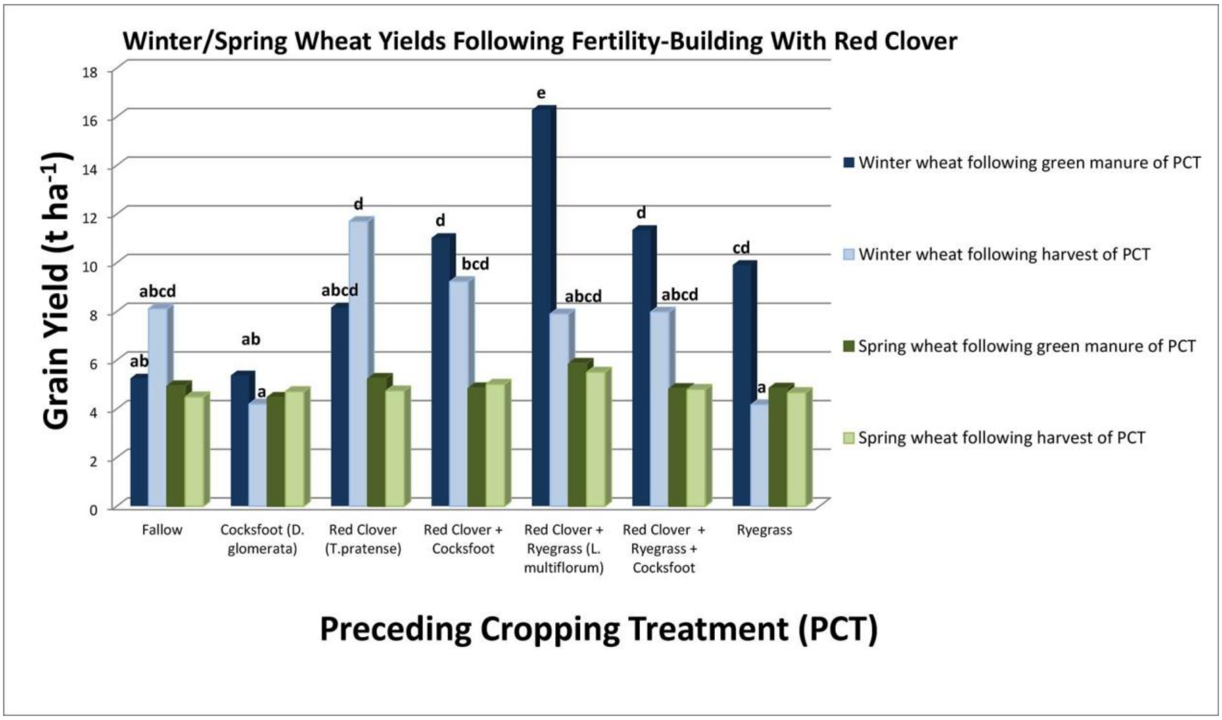

Fig. 2. Impact of various RC combinations under diﬀ ering management strategies on spring/winter wheat over 16 months (winter wheat) and 20 months (spring wheat) (Moyo et al., 2015a,b).
Fallow results indicated control plots with natural regeneration. Bars with the same letters are not signiﬁ cantly diﬀ erent ($P < 0.05$). No signiﬁ cant eﬀ ects on spring wheat were observed

Table 2
Summary of estimated N inputs to global agricultural soils from diﬀ erent studies. Nitrogen Input MT yr^{-1} (MT = 10^6kg)

Reference	(Liu et al., 2010)							(Leip, 2011)	(Smil, 1999b)
Region	Asia	Africa	Europe	North America	South America	Oceania	Global	Europe	Global
Atmospheric Deposition	9.44	0.93	2.02	1.36	0.68	0.04	14.47	2.06	20
BNF	9.66	2.93	1.67	4.38	3.31	0.32	22.27	1	20
Fertilizer	41.1	2.16	8.38	12.17	3.02	1.03	67.84	11.42	80
Manure	9.47	1.29	1.66	2.31	2.40	0.21	17.34	7.07	18
Residues	3.03	1.03	1.48	4.75	0.88	0.19	11.37	3.94	16

3 and 4 summarize the results of these ﬁ ndings, along with the relevant crop, location, weather conditions and measurement methodology in- formation. Measurement methodologies are brieﬂ y explained in Sec- tions 5.1.1 and 5.1.2.

5.1. Nitrogen ﬁ xation assessment methodology

5.1.1. Diﬀ erence method

Total legume N cannot be used to assess ﬁ xation, as some propor- tion of this will always be derived from the soil, not the atmosphere. The diﬀ erence method uses the N yields of non-ﬁ xing reference crops such as grasses, usually grown in adjacent plots, to correct for this. The N derived from atmosphere (Ndfa) is then calculated as the diﬀ erence between reference crop N and ﬁ xing crop N.

5.1.2. ^{15}N methods

N_2 occurs in two isotopes, ^{14}N and ^{15}N. The overwhelming majority of atmospheric N is ^{14}N (96.63%), whilst the remaining 0.36% is ^{15}N (Mariotti, 1983). Soil mineral nitrogen tends to be slightly higher in ^{15}N due to isotopic discrimination – the ^{15}N isotope is heavier than the ^{14}N and its mineral forms undergo denitriﬁ cation and volatilization more slowly. Legume crops will take much of their N from ﬁ xation given the right parameters (rhizobia presence, adequate growth conditions etc) and will therefore be slightly higher in ^{14}N than non-fixing crops. Two methods of measuring fixation are drawn from these principles; natural abundance and isotopic dilution.

5.1.2.1. Natural abundance. The natural ^{15}N enrichment of soil can be used to assess nitrogen fixation. Differences in ^{15}N content in legumes and non-fixing reference crops grown in soil with a known ^{15}N content can be used to calculate Ndfa in legumes.

5.1.2.2. Isotopic dilution. The $^{14}N - {}^{15}N$ ratio of soil mineral nitrogen can be altered using fertilizers enriched with ^{15}N. A non-fixing reference plant grown in conditions in which its sole N source is ^{15}N fertilizer will contain only ^{15}N in its biomass. A fixing plant grown in the same conditions will contain less ^{15}N, as some will be atmospherically derived ^{14}N. This difference can be used to calculate the proportion of Ndfa in legumes .

5.2. Contribution of fixed N to soil and subsequent crop

N fixation measurement methodologies vary in detail and accuracy. Detailed review of their technicalities, merits and limitations can be

Table 3

Summary of nitrogen fixation using difference method.

Location	Cropping System	Reported N Fixation (kg ha^{-1} yr^{-1})		Precipitation (mm)	Reference
UK	RC	241.3		531	(Moyo, 2014)
UK	RC/grass	225.4		531	(Moyo, 2014)
UK	RC/grass	Year 1	37	1056	(Hatch et al., 2007)
UK	RC/grass	Year 2	120	1056	(Hatch et al., 2007)
UK	RC/grass	Year 1	184	749	(Hatch et al., 2007)
UK	RC/grass	Year 2	213	749	(Hatch et al., 2007)
Germany	RC	~ 355		747	(Loges et al., 1999)
Germany	RC/grass	~ 345		747	(Loges et al., 1999)
Germany	RC/grass	175		321	(Gierus et al., 2012)
Belgium	RC/grass	256–400[1]		Not Given	(Deprez et al., 2004)
USA	RC/grass	Year 1	98.7	1450	(Ashworth et al., 2015)
USA	RC/grass	Year 2	246.6	1450	(Ashworth et al., 2015)
USA	RC/grass	Year 1	103.2	1190	(Ashworth et al., 2015)
USA	RC/grass	Year 2	64.4	1190	(Ashworth et al., 2015)
USA	RC	Year 1	110–149[1]	1095	(Sparrow et al., 1995)
USA	RC	Year 2	16–35[1]	1095	(Sparrow et al., 1995)
USA	RC	Year 1	63–83[1]	862	(Sparrow et al., 1995)
USA	RC	Year 2	2–33[1]	862	(Sparrow et al., 1995)

[1] Study reported this range using several different non-fixing reference crops.

found in Peoples et al. (1989) and Ledgard and Steele (1992). The ^{15}N methods are considered more accurate and therefore more widely used than the difference method (Peoples et al., 2015), however all values reported should be considered estimations.

BNF assessments may predict N contributions to subsequent crops. The range documented in Tables 3 and 4 is broad, as is that in similar reviews (Carlsson and Huss-Danell, 2003). This precludes robust statistical analysis, but the key limitation of these findings is they only account for N contained within the aboveground herbage. Accumulation of DM and N in aboveground herbage is thought to be the main driver of BNF in RC cultivation (Dhamala et al., 2017), but belowground N remains unaccounted for when using this proxy. This oversight is compounded by the usual RC management strategy, is mostly to harvest for silage or hay. Mulching is also practiced, but this can inhibit subsequent fixation and regrowth (Moyo, 2014). There are also concerns of N losses through leaching and denitrification with RC green manures (Schmidt et al., 1999). If RC management is mostly to cut forage, then the challenge for agronomists is to translate fixation assessments obtained from aboveground biomass into a realistic estimate of the N contribution to the subsequent crop.

This contribution is made through the senescence of the root system (which contains a store of N) and deposition of N in the rhizosphere (Russell, 1973). Difficulties in defining the extent of the rhizosphere and in recovering entire root systems for analysis make this

Table 4

Summary of nitrogen fixation using ^{15}N methods.

Location	Cropping System	Reported N Fixation (kg ha^{-1} yr^{-1})		Measure of fixed N Methodology	Precipitation (mm)	Reference
USA	RC/wheat intercrop	53.1		^{15}N abundance	429	(Snapp et al., 2017)
USA	RC/wheat intercrop	35.4		^{15}N abundance	780	(Snapp et al., 2017)
Denmark	RC	506		^{15}N abundance	~ 450	(Dhamala et al., 2017)
Denmark	RC	400		^{15}N abundance	Not Given	(Dhamala et al., 2016)
USA	RC/grass	Year 1	106.8	^{15}N dilution	1450	(Ashworth et al., 2015)
USA	RC/grass	Year 2	85.9	^{15}N dilution	1450	(Ashworth et al., 2015)
USA	RC/grass	Year 1	73.7	^{15}N dilution	1190	(Ashworth et al., 2015)
USA	RC/grass	Year 2	80.7	^{15}N dilution	1190	(Ashworth et al., 2015)
Sweden	RC/grass	42.5–59.9[2]		^{15}N dilution	571	(Huss-Danell et al., 2007)
Sweden	RC/grass	19.5–42.1[2]		^{15}N abundance	571	(Huss-Danell et al., 2007)
USA	RC	33		^{15}N abundance	880	(Schipanski and Drinkwater, 2012)
USA	RC/grass	48		^{15}N abundance	880	(Schipanski and Drinkwater, 2012)
Denmark	RC	125		^{15}N dilution	766	(Li et al., 2015)
Denmark	RC/grass	128		^{15}N dilution	766	(Li et al., 2015)
USA	RC/grass	Year 1	152.6	^{15}N dilution	Not Given	(Farnham and George, 1993)
USA	RC/grass	Year 2	92.9	^{15}N dilution	Not Given	(Farnham and George, 1993)
Denmark	RC/grass	357		^{15}N dilution	627	(Rasmussen et al., 2012)
Switzerland	RC/grass	89		^{15}N abundance	791	(Oberson et al., 2013)
USA	RC	Year 1	102–128[1]	^{15}N dilution	1095	(Sparrow et al., 1995)
USA	RC	Year 2	36–44[1]	^{15}N dilution	1095	(Sparrow et al., 1995)
USA	RC	Year 1	75–83[1]	^{15}N dilution	862	(Sparrow et al., 1995)
USA	RC	Year 2	32–33[1]	^{15}N dilution	862	(Sparrow et al., 1995)
USA	RC	Year 1	65–76	^{15}N dilution	Not Given	(Heichel et al., 1985)
USA	RC	Year 2	34.9	^{15}N dilution	Not Given	(Heichel et al., 1985)
USA	RC	Year 3	48.4	^{15}N dilution	Not Given	(Heichel et al., 1985)
USA	RC	Year 4	68.3	^{15}N dilution	Not Given	(Heichel et al., 1985)
Denmark	RC/grass	~ 35		^{15}N dilution	370	(Pirhofer-Walzl et al., 2012)
Sweden	RC	231		^{15}N dilution	525	(Dahlin and Stenberg 2010a)
Sweden	RC/grass	238.6		^{15}N dilution	525	(Dahlin and Stenberg 2010a)

Study reported this range using several different non-fixing reference crops.

[2] This range was reported over three sites in this study.

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contribution difficult to predict (Hogh-Jensen and Schjoerring, 1997). Consequentially there is limited information on the belowground N contributions in RC leys, and this question the merit of BNF assessments based on aboveground herbage, particularly when it's removed. RC is known to grow a large taproot which can grow to a depth of 1 m in the soil (Boller and Nosberger, 1987) which means estimates based on aboveground herbage may underestimate the whole-plant N contribution to the cropping system.

An attempt to account for this has been made by Unkovich et al. (2010), who proposed knowledge of the above/below ground N partitioning can be used to develop a 'root factor', which can be applied to aboveground measurements to estimate N contributions from roots. Peoples et al. (2012) determined aboveground fixed N could be multiplied by a root factor of 1.72 to account for the total plant N contribution (based on a recording of 42% of total plant N contained in the roots). Rhizodeposition of N by RC has also shown to actually exceed the amount of N in the harvested aboveground biomass (Hogh-Jensen and Schjoerring, 2001), meaning estimates of N contributions from RC cover crops omitting this as well as root N may be significant underestimations.

Given the varying crop management and field conditions in which these studies took place, it is difficult to draw definitive conclusions about the volume of N that may be fixed by RC in a given period. Agronomists should not ask how much N will RC fix, but what environmental conditions and management strategies optimize fixation. Widely reported sub-optimal fixation in N-rich environments should be of particular concern (Goh et al., 1996, Stopes et al., 1996) as many farmers will use fertilizers in tandem with RC cultivation. Further research into *Rhizobia* interactions may also facilitate optimal fixation rates in RC cropping.

Most legumes exhibit a degree of specificity in relation to the *Rhizobia* species with which they form symbiosis, and association with a non-specific strain can result in sub-optimal nodule formation and low levels of BNF. RC exhibits a high degree of specificity for the Rhizobia species *Rhizobium leguminosarum* biovar *trifolii* (Taylor and Quesenberry, 1996). Seed inoculation with this biovar can be used to ensure optimal nodulation. This is most important in regions without indigenous *Rhizobium leguminosarum* biovar *trifolii* populations like Latin America (Batista et al., 2015) and Australia, where the *Trifolium* genus is not naturally abundant (Brockwell et al., 1995). Innoculation will not be necessary in areas where this biovar is ubiquitous, for example the UK (Roberts et al., 2017). There is also evidence suggesting within *Rhizobium leguminosarum* biovar *trifolii* there are variations in the strains, some of which will nodulate RC more effectively than others (Miller et al., 2007), and pre-treatment with Nod factors (Rhizobial signalling chemicals) can enhance nodulation (Dominika et al., 2009). These findings indicate how the N contribution of RC can be further optimized through better understanding of the nodulation process and improved inoculation where necessary.

6. Mineralization of nitrogen

N contributions from RC cultivation can be split into two categories: that derived from rhizodeposition during the growth phase (Dahlin and Stenberg, 2010b) and that released upon mineralization of plant residues following termination and cultivation of the subsequent crop (Eriksen, 2001). Rhizodeposition can be broadly defined as the release of organic and inorganic compounds from living plant roots (Gregory, 2006). Rhizodeposition of N is predicted to be higher in legumes than non-legumes because biofixation increases their overall N assimilation rate (Urbatzka et al., 2009).

Estimates of rhizodeposition rates associated with RC leys range from 5% of total plant N (Hammelehle et al., 2014) to over 100% of total plant N (Hogh-Jensen and Schjoerring, 2001). They further range incorporate both field and lab experiments (Kanders et al., 2017), and this method may yet reveal the extent and fate of rhizodeposited N in RC cropping. As with BNF, a broad range is documented, and the ultimately fate of this N is unaccounted for. Some will remain in the soil to be taken up by the subsequent crop, some will be retaken up by the RC crop itself (Janzen, 1990), whilst some more will be lost, either through denitrification (Jensen, 1996) or immobilization by microbial activity (Mayer et al., 2003). This lack of clarity on the amount of N actually deposited and utilized by subsequent crops means the rhizodeposition component of fertility-building with RC leys remains unclear.

Return of organic N to soil is the second component of N cycling in cover cropping. This is known as mineralization and is defined as the decomposition of plant residues into ammonium and nitrate. Traditional agricultural practice favours forage legumes like clovers over grain legumes for this purpose, because they decompose at a faster rate (Peoples et al., 1995). Within the forage legumes, RC has a low C:N ratio range of 13.6-16.7 (Bruulsema and Christie, 1987), which particularly lends itself to rapid decomposition. Grasses typically have higher C:N ratios which delays mineralization. Knowledge of the C:N ratio of cover crops is important when selecting candidate plants for specific purposes. Table 5 summarizes the C:N ratio of RC along with other common cover crops for reference.

The C:N ratio of cover crops may also change over the growing period. Increased lignification associated with maturity tends to raise the C:N ratio and further immobilize N, evidenced by a higher C:N ratio within wheat and oat straw compared to their leaves. A low C:N ratio is desirable for rapid mineralization, however this will not always guarantee N transference. Ensuring efficient N transference in cover cropping with legumes is important in N management (Crews and Peoples, 2005, Snapp and Borden, 2005), and farmers may exploit the changing C:N ratios of maturing RC crops by mulching in the early bloom stage when rapid mineralization rates are required, or at the full bloom stage when slower rates are optimal (Wiersma et al., 1998).

7. Ecosystem services

Agriculture both provides and relies upon ecosystem services (Zhang et al., 2004). RC is known to contribute multiple ecosystem services, which can enhance the sustainability of the overall system. These ecosystem services are summarized in Table 6.

As Table 6 indicates, the ecosystem services associated with RC cropping are mostly soil improvements, although its flowers are also documented to benefit pollinator population and diversity. RC can increase soil organic matter (SOM) which in turn facilitates soil aggregation (FAO, 2014) and water-use efficiency (Thierfelder and Wall, 2009). SOM levels are indicative of soil health are critical for soil quality and function (Varvel, 1994). As a legume, RC is most commonly associated with N contributions, but it is important to note it can also deliver these non-N benefits to the system in which it is cultivated.

Table 5
Summary of C:N ratio of common cover crops (Bruulsema and Christie, 1987; USDA 2011).

across different climates, soil types and measurement methodologies.

Cover Crop	Common Name	Reported C:N Range
Trifolium pratense	Red Clover Leaf	13.7:1
Vicia faba	Vetch Leaf	11:1
Medicago sativa	Alfalfa Leaf	13:1
Avena agrostis	Black Oat Leaf	34:1
Avena sativa	Oat Leaf	42:1
Triticum aestivum	Wheat Leaf	42:1
Secale cereale	Rye Straw	70:1
Triticum aestivum	Wheat Straw	80:1

Some are field-based, others are pot-based. Novel approaches

Table 6
Summary of ecosystem services associated with RC rotations.

Service	Crop	Location	Ref
Increase in soil organic matter	Barley/RC Undersow	Canada	(Angers et al., 1999)
	Pure RC	Estonia	(Lauringson et al., 2013)
	Pure RC	Germany	(Knebl et al., 2015)
Increase in soil microbial activity	Potato/Barley/RC rotation	Canada	(Carter et al., 2009)
	Pure RC	Canada	(Lupwayi et al., 1998)
	Pure RC	Canada	(Drury et al., 1991)
	Small grain/RC undersow	USA	(King and Hofmockel 2017)
Increase in soil aggregation	RC	Denmark	(Miller and Dick 1995)
	Wheat/RC undersown & Barley RC Undersow	Canada	(Raimbault and Vyn 1991)
	Barley/RC undersow	Canada	(Carter and Kunelius 1993)
	RC/ryegrass	Denmark	(Stokholm 1979)
Soil Carbon Sequestration	Barley/RC Undersow	Canada	(Yang and Kay 2001)
	Barley/RC Undersow	Canada	(Meyer-Aurich et al., 2006)
	Wheat/RC Undersow		
	Pure RC	Canada	(Soon and Clayton 2002)
Phytoremediation	Mixed clover (*T. pratense*, *T. repens*, *T. ladino*)	USA	(Dominguez-Rosado and Pichtel 2004)
	Pure RC	Lithuania	(Kackyte et al., 2011)
Provision of food for pollinators	Pure RC	Denmark	(Dupont et al., 2011)
	Pure RC	Chile	(Ruben Palma et al., 2005)
	Pure RC	Germany	(Diekotter et al., 2010)
Increase in soil porosity	Pure RC	UK	(Papadopoulos et al., 2006)
	Pure RC	Canada	(Drury et al., 1991)

8. Weed control

RC cropping may also control weeds. Suppression of weeds will always be desired of cover crops (Liebman and Dyck, 1993), particularly in the UK, where blackgrass (*Alopecurus myosuroides*) populations are becoming increasingly problematic (Chauvel et al., 2001). Predicted yields of 10–15 t DM ha^{-1} for RC crops will likely be capable of weed suppression (Elsaesser et al., 2016, Nagibin et al., 2016), but this can be enhanced by the exudation of phytotoxic chemicals released by the crop itself, a process known as allelopathic weed control (Liebman and Sundberg, 2006).

Studies on allelopathy in RC cropping are summarized in Table 7. Most of these studies were field-based, however some were lab-based (Liebman and Sundberg, 2006, Liu et al., 2013). Potential candidates for the hypothesized allelopathic chemicals were suggested, e.g. phenols in Ohno et al. (2000); Ohno and Doolan (2001) and isoflavonoids in Liu et al. (2013). Maiksteniene et al. (2009) was the only study to observe an increase in weed biomass following a RC/vetch mixture, but this was attributed to an increase in soil tilth and fertility. Although these studies suggest that RC has the capacity to control weeds with allelopathic chemicals in field studies, it still remains unknown if this control is caused by allelopathy or just the physical presence of the crop/residues. However, efforts to understand the physiological basis for weed suppression and allelopathy in cover crops remain important. If allelopathic chemicals can be verified and identified breeders may perhaps begin to increase their expression in breeding programs, and make contributions to sustainability by reducing the need for additional tillage and/or herbicide applications.

RC green manure was shown to have no significant inhibtory effect on maize growth by Conklin et al. (2002) – see Table 7. This finding, and the common practice of undersowing RC into cereals (Koehler-Cole et al., 2017) implies the risk of allelopathic effects on crop plants is non- significant. A review by Wyngaarden et al. (2015) cited some studies in which stunting was initially observed in cereals following RC green manure, but showed these effects did not significantly curtail yield. The nitrogen contribution and soil improvement through RC green manure was thought to outweigh any initial inhibition in growth caused by the exudation of allelopathy residues.

9. Variety selection

RC is a bee-pollinated plant with a gametophytic self-incompatibility system (Taylor et al., 1995), a profile which typically causes intra-specific diversity. This has been consistently observed in RC wild populations and ecotypes (Dias et al., 2008, Pagnotta et al., 2011) and confers diversity to the end products of breeding programs. Three classifications of RC exist, early or late flowering, erect or prostrate and diploid or tetraploid. Characteristics of these varieties and agronomic consequence are reviewed here.

Table 7
Summary of reported weed control by RC in field and laboratory conditions.

Crop	Environment	Effective Weed Control Reported	Weed Species	Reference
RC extract	Laboratory	Yes	18 weeds and 44 crop plants	(Liebman and Sundberg 2006)
RC extract	Laboratory	Yes	*A. thaliana*	(Liu et al., 2013)
RC	Field	Yes	*S. arvensis*	(Ohno and Doolan 2001)
RC	Field	Yes	*S. arvensis*	(Ohno et al., 2000)
RC	Field	Yes	*B. kaber*	(Conklin et al., 2002)
		No	*Z. mays*	
RC	Field	Yes	*L. aplexicaule, P. rhoeas, S. arvensis, C. recutita, P.minor*	(Bilalis et al., 2009)

RC/Italian Ryegrass	Field	No	Unspecified annuals and perennials	(Boguzas et al., 2010)
Wheat/RC undersow	Field	Yes	*S. fanberi*	(Davis and Liebman 2003)
RC Wheat/RC-Italian Ryegrass undersow	Field	No Yes	*S. media, G. aparine, F. convolvulus, T. perforatum, V. arvensis, G. aparine, E. repens*	(Maiksteniene et al., 2009)

9.1. Early and late flowering

The most well-known know classification of RC varieties is early and late flowering (Frame et al., 1998). Terms such as medium/double cut (early) and mammoth/single cut (late) are used in North America to describe the same classification (Taylor and Quesenberry, 1996). Early varieties flower 1–2 weeks before late varieties and have the agronomic consequence of providing more vigorous regrowth than late varieties (Madill and Skepasts, 1981). Late varieties give most of their annual yield at the first cut and will regrow with less vigour than early vari- eties. Some authors cite late varieties as storing more reserves in the root system (Vleugels, 2013), which may also be of agronomic sig- nificance. Classification is more spectrum than dichotomy and some varieties could be described as 'intermediate', i.e. falling somewhere between early and late classification. The literature comparing RC varieties is almost exclusively focused on disease resistance and yield, and the comparative performance of early and late varieties in fertility-building remains largely unstudied.

9.2. Erect and prostrate

RC naturally exhibits an erect growth habit (Bowley et al., 1984) but advances in plant breeding have succeeded in creating prostrate varieties, which produce stolons and can root at auxiliary nodes under optimal conditions (Rumball et al., 2003). These varieties were initially created in Australia and New Zealand by breeders seeking to increase the persistence of RC in response to grazing (Wrightson, 2015). Prostrate varieties can persist in the sward like white clover (Abberton and Marshall, 2005) but also deliver the high-protein forage RC is known for (Lee, 2014). Prostrate varieties are not commonly grown outside of Australia and New Zealand, although European breeders have created one prostrate variety named 'Pastor' (Boller et al., 2012). A stoloniferous growth habit may predict increased persistence, but public sector research confirming this is scarce. The response of prostrate varieties to cutting as opposed to grazing also remains unknown. Vleugels (2013) investigated whether a prostrate growth habit caused resistance to clover rot (*Sclerotinia* sp) using the prostrate varieties Astred, Crossway and Broadway, but found no correlation between growth habit and disease resistance.

9.3. Diploid and tetraploid

RC is a natural diploid (2n = 14), but artificial tetraploid varieties (4n = 28) have been created by breeders (Evans, 1954). Improved agronomic performance in RC tetraploids is predicted by some because natural tetraploidy in angiosperms is often associated with adaptation to adverse environmental conditions (Fawcett and Peer, 2010). Polyploidy occurs naturally in angiosperms through a process known as whole genome duplication (WGD), in which unreduced gametes are produced due to errors in meiosis. Why this happens is unclear, but the subsequent evolutionary success of polyploid populations indicates polyploidy provides a mechanism for speciation and adaption to new environments (Ramsey and Schemske, 2002). The potential adaptive advantages associated with polyploidy are thought to be the driving forces underpinning their evolutionary success (Renny-Byfield and Wendel, 2014, Tang et al., 2014).

Advantages of polyploidy include higher levels of biomass accumulation due to larger polyploid cells (Knight and Beaulieu, 2008) and increased pest resistance (Nuismer and Thompson, 2001). This raises the question; given some of the observed consequences of polyploidy in angiosperms, could tetraploid varieties of RC exhibit desirable morphology and performance? If tetraploidy is found to impart traits that enhance sustainability, then this could open up new avenues to plant breeders developing new cultivars, as well as further options for farmers deciding which varieties to use in fertility-building.

9.3.1. Cell size, biomass accumulation and agronomic consequence

Tetraploid RC varieties produce significantly heavier seeds than diploids (Taylor and Quesenberry, 1996). Heavier seeds produce seedlings with more energy reserves, which means tetraploid RC varieties often grow more vigorously and establish better than diploid varieties. It also means tetraploids may be preferable for farmers sowing directly into mulched residues. However, it must be noted that correspondingly higher sowing rates may also be necessary, which may offset a favourable emergence rate (Taylor and Quesenberry, 1996). This disadvantage can be compounded by poor seed yield associated with tetraploid varieties (Boller et al., 2010), and this adds to estab- lishment costs.

The seed yield deficiency of tetraploid RC is a major constraint in breeding, and if it could be overcome then higher yielding and more disease resistant varieties could be more successfully adopted (Taylor, 2008a). Documented attempts to increase seed yield in RC have in- cluded boron and cobalt applications (Stoltz and Wallenhammar, 2014, Tomic et al., 2014) and the use of marker assisted selection and quantitative trait loci analysis in breeding have also been cited as po- tential tools for identifying methods to improve seed yield (Herrmann et al., 2006, Vleugels et al., 2014). Investigations into the molecular basis of seed-setting in tetraploid RC remain ongoing at the time of writing, and may yet facilitate breeders in increasing seed yield and making the seed more affordable (Amdahl et al., 2017, Kovi et al., 2017).

Tetraploid RC varieties have been shown to exhibit higher above-ground biomass yields than diploids. For example, McBratney (1980) showed tetraploid cultivars had larger petioles and leaf-areas than diploids. Similarly, tetraploid varieties have been shown to give significantly higher DM yield than diploids (Zuk-Golaszewska et al., 2010), but it was observed that these increases were marred by a reduction in foliage content of P and Ca. This indicates a trade-off may exist between the yield and nutritive value of different varieties, useful knowledge for developing site-specific strategies. For example, high-yielding tetraploids may contribute more to soil fertility through increased SOM contributions, whilst high-nutritive diploids may be preferable for stocked systems that use residues for fodder.

9.3.2. Disease resistance

Directly correlating polyploidy with disease and pest resistance is difficult, but Madlung (2013) suggested polyploidy increases resilience to stressful environmental conditions like disease and pest attack. This can occur through increased allelic diversity (Spurgin and Richardson, 2010), or through supplementary expression of genes related to im- munity through multiple genomes (King et al., 2012). Chromosome doubling in breeding may then enhance and strengthen pre-existing resistance in naturally occurring diploid populations. The main diseases and pests of RC are summarized in Table 8, with information about resistant varieties and their ploidy.

Some pest and disease problems with RC can be managed agronomically. For example clover rot can be mitigated by spring sowing (Jones et al., 2003) or a prophylactic bacterial Biocontrol application (Ohberg and Bang, 2010). Crop rotations are also advised, particularly in root rot (Peters et al., 2003), but the literature suggests breeding for resistance is the most effective strategy (Annicchiarico et al., 2015, Jacob et al., 2015). Improved varieties can vastly improve resistance and if tetraploidy in RC varieties does enhance persistence then breeding programs could incorporate it to produce more disease-re- sistant varieties.

10. Management

10.1. Grass mixtures & monocultures

RC in fertility-building can either be grown in monoculture or with companion grasses. It can also be grown in mixture with other legumes

Table 8
Summary of pests and diseases of RC (main appearing in bold) with reported resistant varieties.

Type	Name	Varieties with Reported Resistance	References
Fungus	Clover Rot (*Sclerotinia trifoliorum & Sclerotinia sclerotiorum*)	Kaive (4n) Vanessa (4n) Milvus (2n) Maro (4n) Tedi (4n) No 292 (2n)	(Mikaliuniene et al., 2015) (Vaverka et al., 2003) (Boller and Nüesch 1995) (Vleugels, 2013)
	Root Rot (*Fusarium* sp)	None reported	
	Southern Anthracnose (*Colletotrichum trifolii*)	Starfire (2n) Pavo (2n) Merula (2n) Larus (4n)	(Jacob et al., 2010) (Schubiger et al., 2003) (Boller et al., 2001)
	Northern Anthracnose (*Kabatiella caulivora*)	Marathon (2n)	(Smith, 1994)
	Powdery Mildew (*Erysiphe polygoni*)	Larus (4n) Freedom (2n) Global (2n)	(Boller et al., 2001;Taylor 2008b; Vleugels 2013)
	Rust (*Uromyces trifolii*)	None Reported	
Virus	Bean Yellow Mosaic Virus (*Potyvirus*)	Arlington (2n) Kenstar (2n) Resizta (4n)	(Smith et al., 1973;Taylor and Smith, 1978;Franova and Jakesova, 2014)
	White Clover Mosaic Virus (*Potexvirus*)	Fresko (4n)	(Franova and Jakesova, 2014)
	Red Clover Mosaic Virus (*Carlavirus*)	Sprint (4n)	(Franova and Jakesova, 2014)
Nematode	Stem Nematode (*Ditylenchus dipsaci*)	PI 271627 (2n) Maris Leda (4n)	(Kouame et al., 1997;Toynbee-Clarke and Bond, 1970)
	Root Knot Nematode (*Meloidogyne sp*)	Barduro (2n)	(Quesenberry and Blount, 2012)
	Clover Cyst Nematode (*Heterodera trifolii*)	Kenland (2n)	(Windham and Lawrence, 1988)
Weevil	Clover Leaf Weevil (*Hypera punctata*)	Mammoth (2n) Lakeland (2n) Dollar (2n)	(Gorz et al., 1975)
Phytoplasma	*Phytoplasma* sp.	None reported	

Table 9
Advantages and disadvantages of RC-mass mixtures.

Advantages		Reference
Yield	Nitrogen fertilizer not required for grass productivity	(Pandey et al., 2017)
	Higher DM yields are generally expected	(Frame et al., 1985)
BNF	Grass competition can increase biofixation via N acquisition	(Loges et al., 1999) (Schipanski and Drinkwater, 2011)
Biodiversity	Provides food for pollinators	(Rundlof et al., 2014)
Soil Cover	Grass soil cover can prevent weed establishment if the legume loses vigour	(Hartwig and Ammon, 2002)
Weed Suppression	More rapid grass establishment can suppress weeds following planting	(Cuttle et al., 2003)
Nutrient Rentention	Mixtures are less susceptible to winter leaching	(HCC, 2014)
Forage quality	Higher sugar concentration in companion grasses can improve forage quality	(Merry et al., 2006)
N uptake	High grass N demand can reduce soil N, which helps RC maintain its presence in the mixture	(McElroy et al., 2017)
Disadvantages		
Competion	More vigorous grass growth can outcompete and reduce RC population	(Haynes, 1980)
Establishment	May underperform compared to monocultures if companion species or planting time is unfavourable	(Murrell et al., 2017)

such as sainfoin and lucerne, a strategy which has become popular in the UK (Wilkinson, 2011). Monocultures and mixtures have their own advantages and disadvantages but these are complicated by environmental interactions. For example, higher N yields are predicted for mixtures (Frame et al., 1985). Monocultures may not take up the same volume of soil N as mixtures, but more N may lost through increased leaching over the winter period (IBERS, 2014), which may offset the initial benefit. The advantages and disadvantages associated with mixtures and monocultures are summarized in Table 9.

RC is most commonly grown with grasses in the UK (Rasmussen et al., 2012). Here RC is more associated with forage production than fertility-building, at least in the conventional sector, and its use in mixture is more likely to reflect the improved forage quality mixtures provide (Sturludottir et al., 2014). Given how the advantages and disadvantages of mixing with grasses are not common to all climates and soil types, and may have complex interactions with eachother, it remains unclear whether mixtures or monocultures are optimal for fertility-building. Farmers should then make the decision to include or omit a grass based on their climate, soil type and nutrition requirement of the desired subsequent crop.

10.2. Cutting

As Tables 3 and 4 indicate, RC crops can be cut and removed or cut and mulched. Cut and remove is primarily associated with forage production (Cassida et al., 2000) and mulching is primarily associated with fertility-building in the organic sector (Dahlin et al., 2011), although both can be practiced in either. Early varieties will generally require two cuts, whereas late varieties may only require one. Superficially, mulching could be viewed as best practice for fertility-building, as it returns N to the system, but mulching can limit subsequent regrowth (Moyo et al., 2011) which can limit future N contributions from biofixation. Mulches themselves are also subject to leaching (Bergström and Kirchmann, 2004), which is undesirable both for subsequent soil fertility and environmental integrity. Both cut and remove and cut and mulch are practiced by farmers in fertility-building in RC, but the decision over which to employ is more likely to be determined by individual needs rather than what is optimal, e.g. if forage is required then cuttings must be removed, if not they may be mulched.

10.3. Cultivation

The termination of RC crops and soil preparation for subsequent cultivation is known as green manuring (Repsiene and Skuodiene, 2008). Green manuring is an important component of fertility-building with RC, as it contributes N-rich aboveground biomass and maintains SOM levels (Bath and Elfstrand, 2007). The effect green manuring has on subsequent crop production is widely variable, as the mineralization required for nutrient release is heavily dependent on plant residue quality (C:N ratio, lignin/polyphenol content etc) and soil/weather conditions (Kumar and Goh, 2000). It is also possible to assess the amount of N transferred to subsequent crops by extending the BNF

Table 10
Net Profit of RC silage in four European countries (Doyle and Topp, 2002).

Silage Crop	Net Profit of Silage Produced (€ ha⁻¹)			
	UK	Germany	Sweden	Finland
Pure Swards				
Red Clover	292.1	258.4	220.2	143.6
White Clover	177.9	78.3	109.9	13.5
Lucerne	206.6	151.7	68.6	−37.5
High-Legume Silages[1]				
Red Clover	224.7	203	285.6	181.3
White Clover	164.8	100.5	255.1	92.9
Lucerne	129.0	146.7	141.5	2.85
Low-Legume Silages[2]				
Red Clover	163.5	186.32	244.7	115.4
White Clover	116.7	85.1	227.7	44.3
Lucerne	96.7	146.7	104.7	−37.5

[1] 70% Clover – 30% Grass.
[2] 40% Clover – 60% Grass.

assessments outlined in Section 5 to subsequent crops, i.e. examine their $^{14}N/^{15}N$ ratio and determine how much was legume (and therefore atmosphere) derived (Li et al., 2015). This method gives an accurate account of the N legacy of fertility-building with RC and future studies using it may reveal optimal management strategies in the cultivation of proceeding crops.

Herbicides can also be included in RC termination, either instead of or in tandem with tillage. This is only permissible in the conventional sector and mostly associated with farmers practicing conservation agriculture (Bajwa, 2014). Herbicide use may even be essential in systems utilizing reduced tillage as some authors describe the long RC taproot as difficult to terminate without deep tillage (Curell, 2011). More rapid mineralization rates would be predicted for systems using herbicide application, and this may have significant effects on the expected yields of subsequent crops, but given how these effects are influenced by soil, climate, plant quality and complex interactions between all three, they are difficult to predict.

11. Economic considerations

Economic considerations can be the dominant factor influencing the adoption of new technologies and management practices. If agriculture is to be truly sustainable then productivity and environmental integrity must be profitably maintained. Legumes can offer farmers substantial economic returns and these returns are generally conceived of in direct ways such as the value of silage, reductions in fertilizer/fodder costs and yield increases. The non-marketable impacts of improved soil fer- tility/structure and various ecosystem services (weed control and pol- linator attraction etc) are much harder to quantify (Swinton et al., 2007). The primary use of RC as forage for grazing animals in Europe means that researchers in this region have recognized its value more as silage (Doyle and Topp, 2002). Table 10 summarises their findings on the value difference from growing and feeding a RC instead of grass silage in four European countries. High-legume silages indicate a 70:30 legume:grass ratio and low-legume silages 40:60. Other forages white clover and lucerne are included for comparison. RC is consistently shown to be the most economically viable.

Valuation of RC forage may increase its adoption, as increased demand for livestock products is predicted for the coming decades (Swain et al., 2018), and forage production must increase to meet this. Cuttings can also be valued as both forage and mulch (Arlauskiene et al., 2017), which may allow farmers to use to both ends in the most efficient manner. Breeding in North America has also reduced the drying time of RC forage by removing its stem hairs (Taylor, 2008b), and incorporation of this into other breeding programs, particularly in areas in which drying times are problematic, may alleviate this concern. Increased expression of polyphenol oxidase (PPO) should also be a key breeding target, as PPO activity in ensiling inhibits proteolysis, enhancing N-use efficiency in ruminants and reducing losses in excreta (Hart et al., 2016). The forage value of new varieties is further increased by im- provements in persistence, as these can produce high protein yields for over three years (Marshall et al., 2017), which requires consistent amounts of N from BNF, ultimately contributing to soil fertility.

Difficulties in determining the economic efficacy of RC rotations may restrict their reprisal. External political factors may impose further restrictions. This review has argued that cheap N fertilizer in the post- war era favoured agricultural practices based on mineral input, but other factors have also contributed. Most significant is the replacement of these traditional rotations by more intensive production systems (Rochon et al., 2004). These replacements are often soy and maize rotations cultivated for biofuel production (Lal, 2005). The dis- articulation of livestock from arable production also restricts the adoption of RC/cereal rotations (Weis, 2013), and reintegrating live- stock into arable systems may make RC cultivation more attractive to arable producers.

As this review has outlined, contributions to soil fertility and crop resilience made by RC can be difficult to predict. Such predictions are even more challenging to translate into financial returns, but the cost of business as usual is much easier to quantify (Struik and Kuyper, 2017). The price of fertilizer is linked to the price of fossil fuel energy, which is subject to volatile market forces and may ultimately be unsustainable. This cost is compounded by potential environmental and ecological damage associated with fertilizer-dependent industrial agriculture. Poor, populous and land-scare nations may never sustain themselves without mineral fertilizers (Smil, 1999a), but increased adoption of leguminous crop rotations could still reduce reliance on them, and make significant contributions to sustainable intensification.

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