Red clover (Trifolium pratense) in conservation agriculture: a compelling case for increased adoption

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Introduction

The human population has more than doubled in the past 50 years (UN, 2014). This growth is predicted to reach 9.6 billion by 2050, and it is estimated this will necessitate a 70–100% rise in food production (Godfray et al., 2010). The challenge is considerable, but Malthusian alarmism should not blind us from the crux of the issue. Global food production has grown dramatically over the last 30 years, and has outstripped population growth (FAO, 2010b) but the margin continues to shrink and 795 million people remain food insecure (FAO, 2015). Global considerations also fail to embrace acute food insecurity in particular regions, such as in sub-Saharan Africa and South Asia. Overemphasizing productivity can also obscure the ecological and social dimensions of ensuring global food security (Swaminathan, 2006), and this is perhaps the strongest criticism of previous agricultural innovation. The goal for the twenty-first century should be to increase productivity without expanding arable land, whilst maintaining environmental integrity and public health (Godfray & Garnett, 2014; Tilman, Cassman, Matson, Naylor, & Polasky, 2002). To produce more with less using sustainable practices remains the key challenge facing farmers and agricultural scientists. As outlined by Beddington (2011), sustainable intensification, coupled with the resolution of food access/distribution inequalities, must be achieved if we are to reduce the number of people who remain food insecure (Loos et al., 2014).

The conclusions of Beddington (2011), and the ‘100 questions’ of Pretty et al. (2010) lay out the complexity of achieving this goal. Innovation in a wide variety of agricultural systems from crop rotations, agroforestry, mixed crop-livestock and crop-aquaculture systems to

minimum tillage and precision agriculture will be increasingly required. These strategies will need to be carefully designed, locally adapted and appropriate to specific agroecological/socioeconomic contexts. They will also need to be developed in a farmer-inclusive and participatory manner to ensure they can be successfully adopted. Wheat, rice and maize supply over 50% of global calories (Cassman, 1999) and their future production must continue in a sustainable manner if they are to continue feeding the planet. Cereal production was dramatically increased in the twentieth century using Green Revolution technologies but this sometimes came with associated environmental damage, most prominently reported in Asia (Pingali, 2012; Singh, 2000). The Green Revolution agricultural model of modern crop variety adoption supported by intensive irrigation and substantial chemical-use may be increasingly incompatible with sustainable intensification. If food security is to be achieved in the future then more cereals must be produced through greater optimization of external inputs, in systems that can also prioritize environment and soil conservation (Pretty et al., 2010).

The contribution that red clover (*Trifolium pratense* L.) makes to sustainability in cereal production with particular reference to conservation agriculture is considered in this review paper. Historically red clover (RC) was traditionally cultivated in rotations with other crops to build soil fertility, but the advent of nitrogen fertilizers in the twentieth century has displaced much of this benefit. Clearly, RC still has a role in many organic farming systems (Nykanen, Granstedt, Laine, & Kunttu, 2000) but most of its modern usage now lies in grass/clover leys for forage, including organic systems. As a result, there exists a rich literature on its role in stocked systems. For example; persistence in swards (Abberton & Marshall, 2005; Frame, 1976), the feed-quality of silage (Owens, Albrecht, Muck, & Duke, 1999), the effect of polyphenol oxidase on protein digestibility (Eickler, Gierus, Kleen, & Taube, 2011; Sullivan & Hatfield, 2006) and the potential for bloating caused by phytoestrogen content (Moorby, Fraser, Theobald, Wood, & Haresign, 2004). In this paper, we recommend greater use of RC as a tool to build soil fertility, which still seems to be under-exploited, and suggest it has much more potential to contribute to sustainable agriculture than current uses provide. Current literature of red clover as a fertility-building crop is reviewed with a particular focus on conservation agriculture, and recommendations on how this use may be better optimized in future are considered.

1.1. Conservation agriculture – a note

Conservation agriculture (CA) is a set of principles and practices promoted as a means of achieving sustainable intensification through soil conservation (FAO, 2008; Hobbs, 2007; Pretty, 2008). CA is succinctly described by Kassam et al. (2012) as the synergistic practices of minimal mechanical soil disturbance and no-tillage seeding, with organic mulch cover and crop diversification. It is defined in more detail by the FAO (FAO, 2015) and has been promoted for decades by an array of organizations such as the UN, the EU, DFID, various CGIAR organizations and numerous NGOs (Anderson & Giller, 2012). It has also attracted a number of criticisms. Most notable are that its perceived yield benefits are overstated (Pittelkow et al., 2015), and that competition for residue-use and prohibitively expensive inputs make it untenable for poor farmers (Giller, Witter, Corbeels, & Tittonell, 2009). Some argue that a lack of clarity on terms used and methodologies also makes some research hard to define and difficult to reproduce (Derpsch et al., 2014). The role that chemical inputs should (or should not) play has also been contested (Sommer et al., 2014; Vanlauwe et al., 2014).

Like many soil management practices, it is also difficult to generalize due to the range of soil type differences and local weather/cropping considerations. CA is not a ‘magic bullet’ for achieving sustainable intensification and global food security, but it can make a significant contribution when adapted and applied to each specific location and farming system. However, the issue of terminology is very important to consider. There are a number of terms related to CA tillage practices in the literature; including conservation tillage, no-till, minimal tillage, non-inversion tillage etc. These terms are clearly defined by Kassam, Friedrich, Shaxson, and Pretty (2009).

2. Red clover – a 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 & Quesenberry, 1996). Cultivation and domestication proceeded over the centuries and landrace populations were established all over Europe. These landraces are now mostly conserved *ex situ* and are used as starting points by modern breeders (Boller, Schubiger, & Koelliker, 2010). The use of RC in Europe became ubiquitous by the sixteenth century (Mousset-Declas, 1992), mainly for its protein-rich fodder and nitrogen (N) contribution in crop rotations. This dual role has been cited by some commentators as having more impact on European agriculture than the introduction of the potato (Fergus & Hollowell, 1960).

Around this time Flemish farmers replaced the fallow periods in their rotations with RC and dramatically increased productivity (Rham, 1860). This rotation of wheat-turnip-barley-RC was pioneered by Charles Townshend, 2nd Viscount Townshend in Britain, and became known as the Norfolk-4 Course Rotation (Knox, Leake, Walker, Edwards, & Watson, 2011), an important development in the Agricultural Revolution of the time. The capacity of RC to increase productivity was also recognized by Thomas Jefferson, who wrote,

> 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

in a letter to a friend in 1817 (Jefferson, 1817). RC was instrumental in arable rotations from this time onwards (Chorley, 1981) and is now found almost globally. Recommended growing conditions are summarized in Table 1.

Biologically available N had previously been a limiting factor in food production for European farmers (Kitsikopoulos, 2004), but clover leys allowed this to be overcome. RC was subsequently taken to the New World by European explorers, and its use became more and more widespread until 1909 when Fritz Haber and Carl Bosch developed their method for producing ammonia industrially (Smil, 1991). This watershed moment for agriculture allowed industrialists to manufacture nitrogenous fertilizer, marking the beginning of greater modern dependency on mineral N and the decline of clover-use (Jenkinson, 2001). The post-war economic boom was marked by a dramatic increase in Haber–Bosch ammonia synthesis (Erisman, Sutton, Galloway, Klimont, & Winiwarter, 2008). Global production rose from 3.7 Mt in 1950 to 85 Mt in 2000 (Smil, 2011) and traditional rotations using RC declined in tandem. Hectarage losses ranging from 30% (Rochon et al., 2004) to 70% (Frame, 1976) have been estimated in the decline of RC growing. Nitrogenous fertilizers became a hallmark of late twentieth century agriculture, and this reliance on mineral inputs has led to the commentary that modern agriculture has become 'the art of turning oil into food' (Foster, Clarke, & York, 2010). Despite this, RC is still used as a fertility-building crop in the organic sector, particularly in Europe (Aamlid, 2002; Cormack, Shepherd, & Wilson, 2003; Nykanen et al., 2000). It is also grown in conventional systems in temperate areas of Japan, Russia, North America, Chile, Brazil and Australia, but its overall use in global agriculture has markedly declined (Taylor, 1985). Sustainable intensification will require sustainable N sources, and a revival of RC and other legumes offers significant potential to make greater contributions towards this goal (Beddington, 2011).

2.1. Prospects for red clover in soil fertility-building

Concerns over the environmental impact of mineral nitrogen fertilizers have led to a resurgence of interest in the use of forage legumes to build soil fertility in rotations with cereal crops (Taylor, 2008a). However, the high N demand of cereals makes their production challenging in lower-input systems (Gooding & Davies, 1997). Timely residual N release (i.e. during the spring growth season) is also essential for cereal production. As RC has been shown to release 40% of total N to the soil under conventional tillage within the first 10 weeks, and 70% under no-till (Lupwayi et al., 2006), this intensity of release can provide sufficient N for cereal production. Doel (2013) investigated the

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

	North America, Europe, Northern China/ Japan, Southern Latin America/Australasia		
Global distribution	Survival range	Optimal range	Frame, Charlton, and Laidlaw (1998)
Soil pH	5.0–8.5	6.0–7.6	Rice, Penney, and Nyborg (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	Duiker and Curran (2007)
Soil salinity	0–1.5 dS/m	0–0.75 dS/m	Rogers 2008
Bioactive compounds	Polyphenol oxidase		Lushcer, Mueller-Harvey, Soussana, Rees, and Peyraud (2014)
	Isoflavonoids (Phytoestrogen)		Boue et al. (2003)

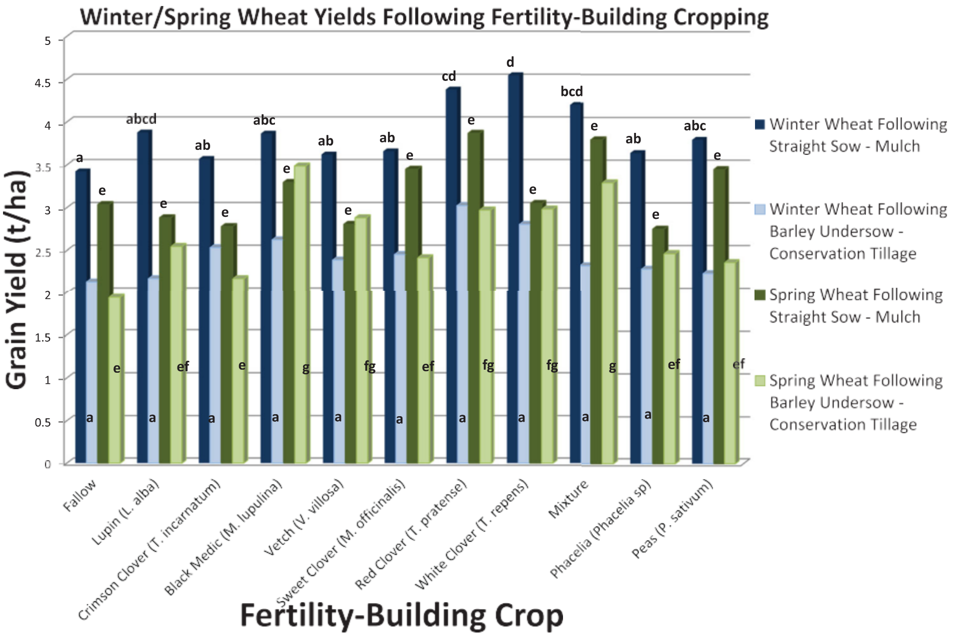

Figure 1. Impact of various fertility-building crops on winter/spring wheat yields over 6 and 12 months with differing management strategies (Doel, 2013) Fallow results indicated control plots with natural regeneration and 'mixture' indicated 40% RC, 30% sweet clover, 15% lupin and 15% black medic. Data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

impact of various fertility-building plants on the yield of subsequent winter and spring wheat in the UK, and highlighted the potential of RC uses (Figure 1).

The results of Doel (2013) indicate RC significantly contributes to soil fertility in short time periods. These significant yield contributions, combined with a reduction in mineral inputs, contribute to the environmental and economic sustainability of cropping systems. Yield benefits associated with RC/cereal rotations have been known for centuries, and the associated ecosystem services they provide have been further explored in modern times but, ultimately, re-uptake has been limited (Schipanski & Drinkwater, 2011). This may be partly due to externalities like government policies, market dynamics or culture (Olmstead & Brummer, 2008), but research into how the yield benefits of RC/cereal rotations can be further optimized could provide a more convincing case for their adoption. Management practices and companion grasses can significant impact on the cereal yield benefits associated with RC rotations (Moorby et al., 2015), and further research into this area will allow farmers to tailor their use of RC to suit their site and climate (Figure 2).

This data indicates that mixtures of RC and companion grasses result in higher subsequent cereal yields than pure swards. Legume/grass combinations can exploit nutrient sources much more effectively than monocultures (Haynes, 1980) and grasses can put an increased demand for fixed N on legumes by competing for rhizosphere N (Cuttle, Shepherd, & Goodlass, 2003; Loges, Kaske, & Taube, 1999). However, these benefits may be cancelled by deleterious competition for water and light from grasses, which can reduce the legume-content and soil fertility-building capacity of the crop (Kleen, Taube, & Gierus, 2011). Both systems are shown to be efficacious in building soil fertility for cereal production, and combined with CA can also contribute to sustainability. This flexibility in use can also allow farmers to tailor their use of RC to suit their own personal needs, i.e. in mixed systems, farmers wishing to use aboveground residues for silage can use a grass mixture without compromising too much on N supply to subsequent cash crops such as cereals (Rochon et al., 2004)

3. Nitrogen for contemporary agriculture

Crop production is by far the biggest human alteration to the global nitrogen cycle, and the supply of N has historically been the most limiting nutrient in global agricultural productivity (Smil, 2002). Many historical

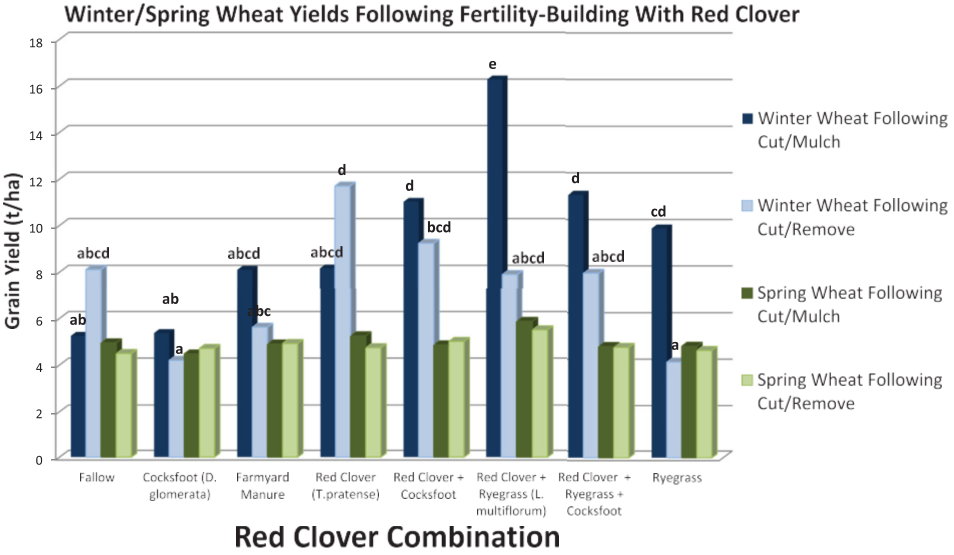

Figure 2. Impact of various RC combinations under differing management strategies on spring/winter wheat over 16 months (winter wheat) and 20 months (spring wheat) (Moyo, Davies, Cannon, & Conway, 2015) Fallow results indicated control plots with natural regeneration. Data sets are averaged and subject to ANOVA, bars with the same letters are not significantly different ($P < 0.05$).

agricultural developments have focused on overcoming this, for example through the use of guano (Hadas & Rosenberg, 1992); transportation of night soil (Petrik, 1954); Chilean saltpetre (Vilches, 2011); traditional recycling of organic wastes (Smil, 2011) and leguminous crop rotations (McNeill & Winiwarter, 2004). Ecologically friendly farming practices such as organic farming prohibit the use of artificial fertilizers to mitigate their associated environmental damage (IFOAM, 2014), but this use is more nuanced in CA systems. The size, climate, budget and management history of any farm will greatly impact how successful the adoption of CA can be, and in some instances, mineral fertilizers may be necessary at least initially (Vanlauwe et al., 2014). However, the preferable supply of more N from leguminous cover crops is ultimately the long-term goal. This strategy is a means to build fertile soils and reduce reliance on mineral inputs for creating more sustainable farming systems.

Although modern conventional agriculture is most associated with Haber–Bosch N, it also utilizes a number of other sources of N. This N is applied to agri- cultural land in crop residues, manure, biofixation, lightening, irrigation and atmospheric deposition (Smil, 1999). Quantification of all these inputs is beset by uncertainties, and the only reliably accurate statistics for agricultural inputs are for mineral fertili- zers; however, some notable estimates have been calculated by Smil (1999) and Galloway et al. (2004). More recently in the European Nitrogen Assessment Project, researchers have made estimates of N cycling in European agriculture using modelling data (Leip, 2011). Estimates of N flow in global agricultural systems have also been proposed in high-resolution maps (Liu et al., 2010).

Knowledge of the various N sources in global agriculture helps to put the contribution of legumes into context, but describing the flow of N in agriculture is difficult for a number of reasons. There are a myriad of uses for crop residues (various fuels, fodders and fibres) and no country keeps comprehensive statistics of their uses. This makes 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 of this 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 widely varying accounts of the contribution of fixation to the overall N economy. These uncertainties make assessing the current and potential N contribution of RC and other legumes crops to global agriculture harder to quantify. Examples of estimated N inputs to global, regional and single-farm agriculture are given in Tables 2 and 3 to contextualize legume contributions.

Table 2. Summary of estimated N inputs to agricultural soils (Liu et al., 2010).

Nitrogen Input MT/yr	Asia	Africa	Europe	North America	South America	Oceania	Global
Atmosph. Deposition	9.44	0.93	2.02	1.36	0.68	0.04	14.47
BNF	9.66	2.93	1.67	4.38	3.31	0.32	22.27
Fertilizer	41.08	2.16	8.38	12.17	3.02	1.03	67.84
Manure	9.47	1.29	1.66	2.31	2.40	0.21	17.34
Residues	3.03	1.03	1.48	4.75	0.88	0.19	11.37

These estimations are both natural and anthropogenic and essential for productivity, but can also drive environmental pollution through eutrophication (Boesch & Brinsfield, 2000; de Vries, Cellier, Erisman, & Sutton, 2011), water quality degradation (Bouraoui & Grizzetti, 2014; Liu, Wu, & Zhang, 2005), and greenhouse gas emissions (Richardson, Felgate, Watmough, Thomson, & Baggs, 2009). Nitrogen pollution occurs through loss of mineral N (leaching); losses from slurry and manure applications (run-off) and the conversion of mineral N to gaseous N by soil microbes (denitrification). The degree to which this occurs is variable, but it can be up to 70% of what is applied (Mulvaney, Khan, & Ellsworth, 2009). These losses can harm the environment whilst limiting the nitrogen-use efficiency of crop plants (Fageria & Baligar, 2005), which means that N supplies for sustainable intensification will need to be more efficient in their transfer to crops, and less carbon-intensive. RC cover crops may simultaneously fix N and mitigate leaching under optimal management strategies (Larsson, Kyllmar, Jonasson, & Johnsson, 2005), which can reduce reliance on inputs and improve the nitrogen efficiency of cereal production systems.

Understanding how sustainable sources of N can be optimized demands an understanding of the biogeochemical cycling of N in systems using legumes. This can be split into three component parts; 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; Cuttle & Goodlass, 2004). Biological nitrogen fixation (BNF) in legumes can be limited or enhanced by soil N status; establishment/persistence, genotypic variation and stresses (Cherr, Scholberg, & McSorley, 2006; Ledgard & Steele, 1992) and mineralization rates by the C/N ratio of crop residues, management strategies, climate and soil microbe activity (Groffman, Hendrix, & Crossley, 1987; Sarrantonio & Scott, 1988). This means that a variety of agronomic factors must be considered for leguminous cover crops to provide sufficient N for cereal production in CA systems.

Table 3. Summary of estimated N inputs to agricultural soils (Allingham et al., 2002; Leip, 2011; Smil, 1999).

	Global (MT/yr) Smil (1999)	EU (MT/yr) Leip (2011)	Typical British farm (kg/ha/yr) Allingham et al. (2002)
Atmosp. deposit	20	2.06	21
BNF	20	1	21
Fertilizer	80	11.42	164
Manure	18	7.07	48
Residues	16	3.94	Not given

3.1. *Biological nitrogen fixation*

A number of studies documenting the volume of nitrogen fixed by RC under varying management strategies have been conducted. Tables 4–6 summarize the results of these findings, along with the relevant crop, location, management, weather conditions and measurement methodology information.

N fixation measurement methodologies vary in detail and accuracy. A detailed discussion of the technicalities, merits and limitations associated with each methodology can be found in Peoples, Faizah, Rerkasem, and Herridge (1989) and Ledgard and Steele (1992). The ^{15}N methods are considered more accurate and therefore more widely used than the difference method, however, Chalk, Inacio, Balieiro, and Rouws (2016) compared records of the 15N abundance/dilution methods and found a probability of .54 that the methods provided estimates within ±10%. Therefore all values reported should be considered estimations. Additionally, all tabulated studies only account for aboveground herbage N. To account for this the ‘root factor’ estimations of belowground contributions are also included, as outlined by Unkovich et al. (2010). All reported estimations have been averaged and are summarized in Tables 4–6.

Measurements of soil N contribution from RC rotations allow farmers to empirically account for N legacies and are, therefore, an important indicator of

Table 4. Summary of nitrogen fixation under cut and mulch management using difference method.

Location	Crop management system	Cropping system	Reported N fixation (kg/ha/yr)	Average (kg/ha/yr)	'Root Factor' consideration (kg/ha/yr)	Measure of fixed N methodology	Weather	Reference
UK	Cut & Mulch	RC/Grass	18/110 175/162	116.25	199.95	Difference	Wet	Hatch, Goodlass, Joynes, and Shepherd (2007)
UK	Cut & Mulch	RC Only	123.4	123.4	212.2	Difference	Dry	Moyo (2014)
UK	Cut & Mulch	RC grass	114.4	114.4	196.8	Difference	Dry	Moyo (2014)
Germany	Cut & Mulch	RC Only	160	160	275.2	Difference	Wet	Loges et al. (1999)
Germany	Cut & Mulch	RC grass	160	160	275.2	Difference	Wet	Loges et al. (1999)
				Mean = 134.8	Mean = 231.9			

Notes: Hyphen indicates range documented, Forward slash indicates multiple years of data cited. Wet refers to a year in which annual precipitation was higher than 550 mm. Dry refers to years lower than 550 mm. 'Root factor' principle outlined in Unkovich, Baldock, and Peoples (2010) and calculated to be 1.72 for RC by Peoples et al. (2012). Average fixation measurements are multiplied by 1.72 to include N contributions from above/below ground biomass.

sustainability. The averages recorded in this data confirm that of other published ranges (Bowley, Taylor, & Dougherty, 1984; Carlsson & Huss-Danell, 2003; Taylor, 1985), who recorded a wide range from 25 to 373 kg/ha/yr for RC. However, the limitation of these studies, and indeed the overwhelming majority of nitrogen fixation studies for forage legumes, is they only account for atmospheric nitrogen contained within the aboveground herbage. The belowground N remains unaccounted for in the majority of cases. This oversight is compounded by the usual RC management strategy, which as Tables 4–6 indicate, is mostly to harvest for silage or hay. Mulching is also practiced, but this can inhibit subsequent fixation and regrowth (Loges et al., 1999; Moyo, Lane, Davies, & Cannon, 2011). There are also concerns of N losses through leaching and denitrification with RC green manures (Schmidt, Philipps, Welsh, & von Fragstein, 1999; Stopes, Millington, & Woodward, 1996), but the use of companion grasses can mitigate this in

Table 5. Summary of nitrogen fixation under cut & remove management using difference method.

Location	Crop management system	Cropping system	Reported N fixation (kg/ha/yr)	Average (kg/ha/yr)	'Root factor' consideration	Measure of fixed N methodology	Weather	Reference
UK	Cut & Remove	RC only	241.3	241.3	173.7	Difference	Dry	Moyo (2014)
UK	Cut & Remove	RC/grass	225.4	225.4	162.3	Difference	Dry	Moyo (2014)
UK	Cut & Remove	RC/grass	37/120 184/213	147.75	106.4	Difference	Wet	Hatch et al. (2007)
Germany	Cut & Remove	RC only	~ 355	~ 355	255.6	Difference	Wet	Loges et al. (1999)
Germany	Cut & Remove	RC/grass	~ 345	~ 345	248.4	Difference	Wet	Loges et al. (1999)
Germany	Cut & Remove	RC/grass	175	175	126	Difference	Dry	Gierus, Kleen, Loges, and Taube (2012)
Belgium	Cut & Remove	RC/grass	256–400	328	236.2	Difference	Not Given	Deprez, Lambert, Decamps, and Peeters, (2004)
Lithuania	Cut & Remove	RC only	231/75/26 216/78/30	109.3	78.7	Difference	Not Given	Kadziuliene (2004)
Lithuania	Cut & Remove	RC/grass	149/182/21 157/182/22	118.833	85.2	Difference	Not Given	Kadziuliene (2004)
USA	Cut & Remove	RC only	110–149/16–35	77.5	55.8	Difference	Wet	Sparrow, Cochran, and Sparrow (1995)
				Mean = 212.3	Mean = 152.8			

Notes: Hyphen indicates range documented, Forward slash indicates multiple years of data cited. Wet refers to a year in which annual precipitation was higher than 550 mm. Dry refers to years lower than 550 mm. 'Root factor' principle outlined in Unkovich et al. (2010) and calculated to be 1.72 for RC by Peoples et al. (2012). Average fixation measurements are multiplied by 0.72 to include N contributions from root biomass and exclude removed cuttings.

Table 6. Summary of nitrogen fixation under cut & remove management using ^{15}N methods.

Location	Crop management system	Cropping system	Reported N fixation (kg/ha/yr)	Average N fixation (kg/ha/yr)	'Root factor' consideration	Measure of fixed N methodology	Weather	Reference
Sweden	Cut & Remove	RC/grass	42.5–59.9	51.2	36.9	^{15}N dilution	Wet	Huss-Danell, Chaia, and Carlsson (2007)
Sweden	Cut & Remove	RC/grass	19.5–42.1	30.8	22.2	^{15}N abundance	Wet	Huss-Danell et al. (2007)
USA	Cut & Remove	RC only	33	33	23.8	^{15}N abundance	Wet	Schipanski and Drinkwater (2012)
USA	Cut & Remove	RC/grass	48	48	34.6	^{15}N abundance	Wet	Schipanski and Drinkwater (2012)
USA	Cut & Remove	RC/grass	152.6/92.9	122.75	88.4	^{15}N dilution	Wet	Farnham and George (1993)
Denmark	Cut & Remove	RC/grass	357	357	257	^{15}N dilution	Wet	Rasmussen, Søegaard, Pirhofer-Walzl, and Eriksen (2012)
Switzerland	Cut & Remove	RC/grass	61–123	92	66.24	^{15}N abundance	Wet	Oberson et al. (2013)
USA	Cut & Remove	RC only	102–128/36–44	77.5	55.8	^{15}N dilution	Wet	Sparrow et al. (1995)
USA	Cut &Remove	RC only	65–34.9–48.4–76	56.075	40.4	15N dilution	Not Given	Heichel, Vance, Barnes, and Henjum (1985)
Sweden	Cut & Remove	RC/grass	201.9	201.9	145.4	15N dilution	Wet	Dahlin and Stenberg (2010)
Denmark	Cut & Remove	RC/grass	~ 35	~ 35	25.2	15N dilution	Wet	Pirhofer-Walzl, Rasmussen, Hogh-Jensen, Eriksen, and Soegaard (2012)
Sweden	Cut & Remove	RC pure	231	231	166.3	15N dilution	Dry	Dahlin and Stenberg (2010)
Sweden	Cut & Remove	RC/grass	250.7	250.7	180.5	15N dilution	Dry	Dahlin and Stenberg (2010)
				Mean = 122.1	Mean = 87.9			

Notes: Hyphen indicates range documented, Forward slash indicates multiple years of data cited. Wet refers to a year in which annual precipitation was higher than 550 mm. Dry refers to years lower than 550 mm. 'Root factor' principle outlined in Unkovich et al. (2010) and calculated to be 1.72 by Peoples et al. (2012). Average fixation measurements were multiplied by 0.72 to include N contributions from root biomass and exclude removed cuttings.

leaching-prone sites and climates (Moyo, Davies, Cannon, & Conway, 2016). If RC management is mostly cut and remove, then the challenge for agronomists is to translate the measurement of fixation obtained from these cuttings into a realistic estimate of the actual N contribution to the soil.

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 contribution difficult to predict (Hogh-Jensen & Schjoerring, 1997). Consequentially there is limited information on the belowground N contributions in RC leys, and this may confound the applicability of N fixation measurements based on aboveground herbage (which is generally used for forage). RC is known to grow a large taproot which can grow to a depth of 1 metre in the soil (Boller & 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 that 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) calculated this to be 1.72 for RC (based on a recording of 42% of total plant N contained in the roots). This consideration is included in Tables 4–6. Hogh-Jensen, Loges, Jorgensen, Vinther, and Jensen (2004) offers a more detailed empirical model for assessing N legacies in clovers, but this model requires soil N details that are often absent in studies documenting N fixation (Table 7).

The data shows what soil-N contributions farmers can realistically expect from 1 year RC crops under differing management strategies. Nitrogen fixation in forage legumes is moisture-sensitive and will be reduced in times of drought (Ledgard, Brier, & Littler, 1987), but the sparsely available studies on dry years with RC make this difficult to assert. As a relatively deep-rooted plant, fixation in RC may be less effected than more shallow-rooted forage legumes, like white clover (*Trifolium repens*). The literature on measurement methodologies also suggests the difference method tends to overestimate fixation (Ledgard & Steele, 1992) and this tendency has been confirmed by the lower recorded measurements for the isotopic methods tabulated in Table 6. However, none of the studies reported in Tables 4–6 account for the rhizode- position of N by RC. Rhizodeposition of N by RC has been shown to actually exceed the amount of N in the harvested aboveground biomass (Hogh-Jensen & Schjoerring, 2001), which means that estimates of N contributions from RC cover crops omitting this may, in fact, be significant underestimations.

However, given the varying crop management and field conditions in which these studies took place, it is quite difficult to draw definitive conclusions about the volume of N that may be fixed by RC in a given period. The important point is not that RC has the capacity to fix a certain amount of N, but that with the right environmental parameters, genetic varieties and management strategies it can then supply sufficient N to subsequent non-fixing crop plants (Bowley et al., 1984; McBratney, 1981). RC has also been shown to be a more efficient fixer than other similar forage legumes like white clover and alfalfa (Frame et al., 1998). This propensity for BNF may be why RC may be more popular with European farmers, particularly in low-input systems that prioritize greater sustainability (Gierus et al., 2012). Some of the literature suggests that fixation rates will not be optimal in N-rich environments (Goh, Mansur, Mead, & Sweet, 1996; Haynes, 1980; Staples et al., 1996; Waterer & Vessey, 1993) as BNF is an energy-expensive reaction and fixation will be reduced if N is readily available. However other studies indicate that fixation is not limited by N-rich soil (Rasmussen et al., 2012; Schipanski & Drinkwater, 2012), which means that RC can also be used in combination with fertilizers if required.

BNF is a two stage process encompassing nodulation followed by N fixation. This subject has been widely studied (Fisher & Long, 1992; Long, 1989; Oldroyd, Murray, Poole, & Downie, 2011). Nodulation occurs following a successful exchange of signalling chemicals between the host and *Rhizobium*, and is dependent on the host plant genotype, the Rhizobia strain and their combined interactions with soil and climate (Bordeleau & Prevost, 1994). Some rhizobia have a very broad host range, for example, the NG345 strain is known to nodulate over 100 genera (Pueppke & Broughton, 1999), while other nodulation (Fisher & Long, 1992).

Rhizobia are much more specific in host selection (Sprent, 1989). 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. This issue must be considered when using legumes for soil improvement. RC exhibits a high degree of specificity for the Rhizobia species *Rhizobium leguminosarum* biovar *trifolii* (Taylor & Quesenberry, 1996), which is known to only nodulate plants of the *Trifolium* genus (Denarie, Debelle, & Rosenberg, 1992). Seed inoculation with this biovar can be used to ensure optimal nodulation. This is most important in regions without indigenous *R. leguminosarum* biovar *trifolii* populations like Latin America (Batista et al., 2015; Santillana, Freire, Sá, & Sato, 1998) and Australia, where the *Trifolium* genus is not naturally abundant (Brockwell, Bottomley, & Thies, 1995). There is also evidence suggesting that within *R. leguminosarum* biovar *trifolii* there are variations in the strains, some of which will nodulate RC more effectively than others (Miller, Elliot, Sullivan, & Ronson, 2007), and that pre-treatment with Nod factors (Rhizobial signalling chemicals) can enhance nodulation (Dominika, Jerzy, Monika, Stefan, & Anna, 2009). These findings indicate how the N contribution of RC can be further optimized through better

Table 7. Summary of means from Tables 4–6.

Table	Mean recorded fixation (kg/ha/yr)	Estimated soil N legacy from 'root factor' calculations (kg/ha/yr)
Table 3 (Cut/Mulch – difference method)	134.8	231.9
Table 4 (Cut/Remove – difference method)	212.3	152.8
Table 5 (Cut/Remove – ^{15}N methods)	122.1	87.9

understanding the nodulation process and chemistry involved, and improved inoculation where necessary.

3.2. Mineralization of nitrogen

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, Ladha, & Herridge, 1995). Within the forage legumes, RC has a relatively low C:N ratio range of 13.6–16.7 (Bruulsema & Christie, 1987), which particularly lends itself to rapid decomposition. Grasses typically have higher C:N ratios which causes N immobilization. This reduces the availability of N to the subsequent crop, but is also the reason grasses are used to prevent leaching (Kuo & Sainju, 1998; Schroder, VanDijk, & DeGroot, 1996). Knowledge of the C:N ratio of cover crops (Table 8) is important when selecting candidate plants for specific purposes.

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 green manuring in conservation agriculture, however, this will not always guarantee N transference. Ensuring efficient N transference in cover cropping with legumes is cited as one of the most important elements of N management (Crews & Peoples, 2005; Snapp & Borden, 2005), and timing of tilling and sowing is also cited as important when optimizing transference of fixed N from legume residues to subsequent crops (Brandsaeter, Heggen, Riley, Stubhaug, & Henriksen, 2008; Francis, Haynes, & Williams, 1995). Other factors include residual biochemical composition (Fox, Myers, & Vallis, 1990; Sarrantonio & Scott, 1988), management strategies (Varco, Frye, Smith, & MacKown, 1989), climatic conditions (Jarvis, Stockdale, Shepherd, & Powlson, 1996) and soil microbial activity (Juma & McGill, 1986). It is difficult to draw direct 'one-for-one' comparisons between RC systems and the mineral inputs of conventional agriculture, or the animal-origin inputs of organic agriculture (Cherr et al., 2006).

Table 8. Summary of C:N ratio of common cover crops (Bruulsema & Christie, 1987; USDA, 2011).

Cover crop	Common name	Reported C:N range
T. pratense	Red Clover	13.7:1
Medicago sativa	Alfalfa	13:1
Vicia faba	Vetch	11:1
Avena sativa	Oat	42:1
Avena agrostis	Black Oat	34:1
Triticum aestivum	Wheat	42:1
Triticum aestivum	Wheat Straw	80:1
Secale cereale	Rye Straw	70:1

There is also a trade-off involved in determining optimal methods of returning mineral nitrogen to the soil. In CA system tillage may initially cause soil compaction in arable fields (Ball, Lang, Robertson, & Franklin, 1994; Salem, Valero, Munoz, Rodriguez, & Silva, 2015) which may reduce the N mineralization rate, but it has also been shown to ameliorate soil compaction in the longer term (Hobbs, 2007; Holland, 2004). However, given RC exhibits relatively high levels of BNF; has a low C:N ratio, and is suitable for cultivation across a wide range of soil pH, it can justifiably be said it is a suitable candidate legume for use in conservation agriculture.

4. Ecosystem services

Agricultural ecosystems both provide and rely upon important ecosystem services (Zhang, Ricketts, Kremen, Carney, & Swinton, 2007). Although CA was initially conceived to mitigate the soil erosion associated with intensive agriculture (Baveye et al., 2011), it also builds sustainability through the provision of multiple ecosystem services (Palm, Blanco-Canqui, DeClerck, Gatere, & Grace, 2014). These services are mostly related to soil structure, efficiency in nutrient cycling and weed suppression (Snapp et al., 2005), but RC can further extend this to include pollinator attraction and phytoremediation of soils. Ecosystem services associated with RC are summarized in Table 9.

4.1. Soil organic matter

One of the ecosystem services of RC cover crops is the increase in soil organic matter (SOM) that can be achieved with appropriate management strategies. This may be optimized through conservation tillage and green manuring (Lal, 2004). SOM levels are indicative of soil health, are frequently used as such and are regarded as critical for soil quality and function (Varvel, 1994). However, SOM is a broad term referring to range of humified and biologically active compounds, including readily decomposable material, plant litter and roots, and dead and living microbes

Table 9. Summary of ecosystem services associated with RC rotations.

Service	Crop	Management	Location	Ref
Increase in SOM	Barley/RC Undersow	Conventional	Canada	Angers, Edwards, Sanderson, and Bissonnette (1999)
	Pure RC	Green manure	Estonia	Lauringson, Talgre, and Makke (2013)
	Pure RC	Mulch	Germany	Knebl, Leithold, and Brock (2015)
Increase in soil microbial activity	Potato/Barley/RC rotation	Conservation Tillage	Canada	Carter, Peters, Noronha, and Kimpinski (2009)
	Pure RC	Green manure	Canada	Lupwayi, Rice, and Clayton (1998)
	Pure RC	Conventional	Canada	Drury, Stone, and Findlay (1991)
Increase in soil aggregation	RC	Green manure	Denmark	Miller and Dick (1995)
	Wheat/RC undersown & Barley RC Undersow	Conservation Tillage	Canada	Raimbault and Vyn (1991)
	Barley/RC undersow	Conventional	Canada	Carter and Kunelius (1993)
	RC/ryegrass	Green manure	Denmark	Stokholm (1979)
Soil Carbon Sequestration	Barley/RC Undersow	Conventional & Conservation	Canada	Yang and Kay (2001)
	Barley/RC Undersow Wheat/RC Undersow	Conventional & Conservation	Canada	Meyer-Aurich et al. (2006)
	Pure RC	Green manure	Canada	Soon, Arshad, Haq, and Lupwayi (2007)
Phytoremediation	Mixed clover (*T. pratense, T. repens, T. ladino*)	Pots in glasshouse experiment	USA	Dominguez-Rosado and Pichtel (2004)
	Pure RC	Pots in glasshouse experiment	Lithuania	Kackyte, Grigiskis, Paliulis, and Aikaite-Stanaitiene (2011)
Provision of food for pollinators	Pure RC	RC grown for seed	Denmark	Dupont, Damgaard, Simonsen, and Stout (2011)
	Pure RC	RC grown for seed	Chile	Ruben Palma, Ramon Rebolledo, Alfonso Aguilera, and Carlos Klein (2005)
	Pure RC	RC cultivated in pots beside oilseed rape	Germany	Diekotter, Kadoya, Peter, Wolters, and Jauker (2010)
Increase in soil porosity	Pure RC	Green manure	UK	Papadopoulos, Mooney, and Bird (2006)
	Pure RC	Conventional	Canada	Drury et al. (1991)

(Gregorich, Carter, Angers, Monreal, & Ellert, 1994) and therefore should not be considered an absolute measure of soil fertility. Levels of soil aggregation are typically increased by high SOM which can reduce susceptibility to erosion, and also contributes to soil health by providing nutrition to soil microbes (FAO, 2014). Cover cropping is a widely used method to increase SOM, although the degree to which the contribution is made will heavily depend upon the species used and environmental factors (Cherr et al., 2006). Maintenance of SOM is imperative if arable land is to be preserved, and intensive farming without continual ground cover or rotations can quickly deplete it. This can be exacerbated by intensive tillage, which may expose SOM to the atmosphere where it can be lost through oxidation (Arshad, Schnitzer, Angers, & Ripmeester, 1990). Pre-existing SOM levels are also an important determinant in the capacity of RC to return SOM to the soil. Griffin and Porter (2004) showed that RC does not contribute significantly to SOM when used as a green manure in soils with high pre-existing SOM, which further indicates that RC is put to best use in less fertile and more exhausted soils.

4.2. Water-use efficiency

High levels of SOM are considered to be key indicators of sustainability within cropping systems (Thierfelder & Wall, 2009). Increasing levels of SOM promote microbial activity, and coupled with conservation tillage this aggregates soil and can encourage root growth. This, in turn, improves agricultural water consumption, ultimately boosting the water-use efficiency (Thierfelder, Amezquita, & Stahr, 2005). Plant biomass and yield are linked to transpiration and the water-use efficiency of a cropping system, which refers to its capacity to convert water into yield (Hatfield, Sauer, & Prueger, 2001; Sinclair, Tanner, & Bennett, 1984). An estimated 80% of global freshwater supplies are used by agriculture, but only half of this supply is efficiently used (Hamdy, Ragab, & Scarascia-Mugnozza, 2003), and scarcity is cited as the most pressing water issue for sustainable agriculture (Jury & Vaux, 2005). Although currently less than half of global food production is irrigation-dependent (Fereres & Connor, 2004), any increase in yield per unit rainfall that can be incurred by agricultural innovation will reduce this dependency. This is of particular importance for sustainable agriculture in drought-prone areas, where drought resilience is a major target in contemporary forage breeding and legume management (Annicchiarico, Barrett, Brummer, Julier, & Marshall, 2015).

An increase in crop water-use efficiency is directly correlated to an increase in SOM, and it has been estimated that every 1% increase in SOM results in a 3.7% increase soil water holding capacity (Hudson, 1994). The correlation of increased SOM with improved water-use efficiency in CA is well documented (Araya, Nyssen, Govaerts, Deckers, & Cornelis, 2015; Erenstein & Laxmi, 2008; Jat et al., 2009), but this is more attributed to management strategies than specific cover crop. Such crops may, however, have morphologies more conducive to increased water-use efficiency than others. RC is known to grow a tap root which can extend up to 1m deep (Bowley et al., 1984), which creates channels for water to penetrate deeper into the soil (Carter & Kunelius, 1993). This, combined with the increase in water retention associated with the incorporated residues in conservation tillage contributes to the increase in water-use efficiency in RC rotations. However, this tap root is known to naturally deteriorate after one year of growth (Kendall & Stringer, 1985) and this is cited as a potential factor contributing to poor drought tolerance and persistence observed in some RC swards. Farmers in drought-prone areas should perhaps consider this before keeping a clover rotation for longer than one year.

4.3. Weed control

Control of weeds in CA systems is a contentious issue. In conventional systems, tillage buries weeds and prepares the seedbed structure (Prihar, Akhtar, & Nizami, 1990), which is essential for subsequent crop establishment and growth (Atkinson, Sparkes, & Mooney, 2007). Conservation tillage may not achieve this and can result in increased weed growth (Bhaskar, Lovera, Davies, & Cannon, 2014). Weed biodiversity can also change dramatically in the conversion to conservation tillage (Froud-Williams, Chancellor, & Drennan, 1981; Froud-Williams, Drennan, & Chancellor, 1983) and this is cited as a major concern for farmers regarding the switch to conservation agriculture (Buhler, Stoltenberg, Becker, & Gunsolus, 1994). The change is thought to occur because weed populations tend to persist longer in CA systems than under conventional tillage. Some researchers have called for more long-term studies in weed control in conservation agriculture for this reason (Swanton & Murphy, 1996). There is a limited amount of study on RC and weed infestations in this area but there has been some research into the role of allelopathy. This use of phytotoxic defence chemicals released by plants for weed control may have potential to also reduce the need for herbicide applications (Liebman & Sundberg, 2006; Singh, Batish, & Kohli, 2003)

Results of studies on the influence of RC cover crops in weed control are reported in Table 10. Most of these studies were field-based; however, some were lab-based (Liebman & Sundberg, 2006; Liu et al., 2013). Potential candidates for the hypothesized allelopathic chemicals were suggested, for examples phenols in Ohno et al. (2000, 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 uncertain if this control is caused by chemical action or other reasons, including the physical presence of residues on the topsoil. 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 then plant breeders can perhaps begin to increase their expression in breeding programmes, and make contributions to sustainability by reducing the need for additional tillage and/or herbicide applications.

5. Variety performance and ploidy

RC is a bee-pollinated plant with a gametophytic self-incompatibility system (Townsend & Taylor, 1985), a profile which typically causes a high degree of intra-specific diversity. This has been consistently observed in RC wild populations and ecotypes (Dias, Julier, Sampoux, Barre, & Dall'Agnol, 2008; Pagnotta, Annicchiarico, Farina, & Proietti, 2011; Ulloa, Ortega, & Campos, 2003) and means that the end products of different breeding programs exhibit high degrees of diversity. RC is a natural diploid ($2n = 14$), but tetraploid varieties ($4n = 28$) have been bred since the fifties (Evans, 1955). Doubling of chromosomes in RC can occur through application of colchicine, nitrous oxide or other chemicals that interfere with chromosome segregation, and create tetraploid varieties

Table 10. Summary of reported weed control influences by RC in field and laboratory experiments.

Crop	Management	Effective Weed Control Reported	Bioassay	Reference
RC extract	Aqueous extract (Shoots)	Yes	18 weeds and 44 crop plants	Liebman and Sundberg (2006)
RC extract	Aqueous extract (Roots)	Yes	*A. thaliana*	Liu et al. (2013)
RC	Green manure	Yes	*S. arvensis*	Ohno and Doolan (2001)
RC	Green manure	Yes	*S. arvensis*	Ohno et al. (2000)
RC	Residues and compost added to soil	Yes	*B. kaber* *Z. mays*	Conklin et al. (2002)
RC	Conventional	Yes	*L. aplexicaule* *P. rhoeas* *S. arvensis* *C. recutita* *P.minor*	Bilalis, Karkanis, and Efthimiadou (2009)
RC/Italian Ryegrass	Green manure	No	Unspecified annuals and perennials	Boguzas, Marcinkevičiene, and Pupaliene (2010)
Wheat/RC undersow	Conventional	Yes	*S. fanberi*	Davis and Liebman (2003)
RC	Green manure	No	*S. media*	Maiksteniene, Arlauskiene, Velykis, and Satkus (2009)
Wheat/RC-Italian Ryegrass undersow	Conventional	Yes	*G. aparine* *F. convolvulus* *T. perforatum* *V. arvensis* *G. aparine* *E. repens*	

(Taylor & Quesenberry, 1996). Improved agronomic performance in RC tetraploids is predicted because natural tetraploidy in angiosperms is often associated with adaptation to adverse environmental conditions. 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 that polyploidy provides a mechanism for speciation and adaption to new, more adverse environments (Mason & Pires, 2015; Ramsey & Schemske, 2002; Thompson & Lumaret, 1992). The potential adaptive advantages associated with polyploidy are thought to be the driving forces underpinning their evolutionary success (Renny-Byfield & Wendel, 2014; Soltis et al., 2009; Tang, Lyons, & Schnable, 2014).

These advantages include higher levels of biomass accumulation due to relatively larger polyploid cells (Knight & Beaulieu, 2008; Stebbins, 1971), hardiness due to their evolutionary connection to adverse environments (Ramsey & Schemske, 1998; Thompson & Lumaret, 1992) and increased pest/disease resistance (Nuismer & Thompson, 2001). The breeding of tetraploid plants has been an effort to input these potential advantages into new varieties for agriculture. RC varieties have traditionally been classified phenologically as early and late flowering, with early varieties, exhibiting two growth flushes and late varieties only one (NIAB, 2014); however ploidy may play an equally significant role in determining agronomic performance. This is an important factor for farmers to consider when differentiating between varieties and raises the question; given some of the observed consequences of polyploidy in angiosperms, could tetraploid varieties of RC exhibit desirable morphology and performance for use in conservation agriculture systems? 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 sow for better soil conservation purposes.

5.1. *Cell size, biomass accumulation and agronomic consequence*

One of the most reliably uniform differences between diploid and tetraploid varieties of RC is seed weight. Tetraploid varieties produce significantly heavier seeds than diploids (Taylor & Quesenberry, 1996). Heavier seeds produce seedlings with more energy reserves, which means that 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 & Quesenberry, 1996). This disadvantage can be compounded by poor seed yield associated with tetraploid varieties (Boller et al., 2010), and this adds to establishment costs. Tetraploids are known to produce fewer seeds and flower heads than diploids (Vleugels, Roldan-Ruiz, & Cnops, 2015), which can make their seed production economically untenable. 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, 2008). Documented attempts to increase seed yield in RC have included boron and cobalt applications (Stoltz & Wallenhammar, 2014; Tomic, Stevovic, Durovic, & Stanisavljevic, 2014) and the use of marker assisted selection and quantitative trait loci analysis (Herrmann, Boller, Studer, Widmer, & Kolliker, 2006; Vleugels, Cnops, & Roldan-Ruiz, 2014) have also been cited as potential tools for identifying methods to improve seed yield in breeding.

The increase in biomass in tetraploid varieties is a hindrance in seed yield, but it can be advantageous if it also occurs in the vegetation of RC tetraploids (Anderson, 1971). When correlated with increased dry matter (DM) accumulation, higher aboveground biomass yield means more carbon and mineral nutrition can be returned to the soil. Tetraploid RC varieties have been shown to exhibit higher aboveground biomass yields than diploids. For example, McBratney (1980) reported that examined tetraploid cultivars had larger petioles and leaf-areas than diploids across a sample of three varieties at both ploidy levels. Similarly, tetraploid varieties have been shown to give significantly higher DM yield than diploids (Zuk-Golaszewska, Purwin, Pysera, Wierzbowska, & Golaszewski, 2010), but it was observed that these increases were marred by a reduction in the mineral content of P and Ca. This indicates that a trade-off exists between the yield and nutritive value of different varieties, and knowledge such as this is useful in developing site-specific strategies. For example, high-yielding tetraploids may contribute more to soil conservation systems through increased SOM contributions, whilst high-nutritive diploids may be preferable for stocked systems that use residues for fodder.

These studies indicate that tetraploids provide greater yields than diploids, but this remains unclear. A comprehensive study involving 93 RC varieties concluded that tetraploidy only resulted in a 6.5% average increase in dry matter yield compared to diploid varieties (Liatukas & Bukauskaite, 2012), which was not considered to be significant. In another study documenting location/cultivar interaction in RC, a 10–60% increase in dry matter accumulation was observed in tetraploid cultivars in humid mountain regions (Leto et al., 2011), but it was thought this was dependent on environmental factors, as performance was not matched in dry lowland areas. The results of these studies suggest that although biomass increases as a result of tetraploidy, this increase may be solely due to increased soil water availability. Therefore, it is also possible tetraploidy in RC may have little impact on DM accumulation, forage quality, and uptake of C and N for return through conservation agriculture.

5.2. Disease resistance

Directly correlating polyploidy with disease and pest resistance is difficult, but Madlung (2013) suggested that polyploidy may contribute to a functional increase in response options to a spectrum of stressful environmental conditions, including disease and pest attack. This can occur through increased allelic diversity, as higher diversity in immunity genes can boost recognition of pathogens (Spurgin & Richardson, 2010), or through the supplementary expression of genes related to immunity through multiple genomes (King, Seppälä, & Neiman, 2012). Vleugels (2013) proposed that polyploidy may contribute to resistance through a broader range of resistance occurrence. RC, as an obligate out-crosser, has mostly heterozygous genotypes. At 50% frequency of a dominant resistant allele, only 75% of a diploid population will contain at least one resistance allele (25% RR, 50% Rr and 25% rr). If this population were tetraploid the number of individuals with a least one resistance allele would increase to 93.75% (as only 6.25% of individuals occur as rrrr). This implies that chromosome doubling in breeding may 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 (Table 11).

Some pest and disease problems with RC can be managed agronomically. For example, clover rot can be mitigated by spring sowing (Jones, Abberton, & Weller, 2003) or a prophylactic bacterial Biocontrol application (Ohberg & Bang, 2010). Crop rotations are also advised, particularly in root rot (Peters, Sturz, Carter, & Sanderson, 2003). However much of the

Table 11. Summary of pests and disease of RC (main appearing in bold) with reported resistant varieties.

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

literature suggests breeding for resistance is the most effective strategy (Annicchiarico et al., 2015; Jacob, Hartmann, Schubiger, & Struck, 2015; Pokorny, Smolikova, & Jakesova, 1995; Skipp & Christensen, 1990). Improved varieties can vastly improve resistance, as seen in the closely related white clover (*T. repens*), which has undergone significant improvement through modern molecular plant breeding (Abberton & Marshall, 2005), RC has not yet been improved to the same extent. This means there is considerable scope for variety-improvement, given how rapidly genomic-based breeding has advanced (Abberton et al., 2015) and how sequencing work has shown RC to be a suitable candidate for association studies and genomic selection (De Vega et al., 2015). There are also large numbers of RC varieties, cultivars, landraces and *ex-situ* germplasm collections available for study. The USDA alone holds an estimated 1367 RC accessions (Smykal et al., 2015), whilst the estimate for *Trifolium* accessions globally is about 74,000 (FAO, 2010a). New varieties with improved disease/pest resistance could vastly improve persistence, which could contribute greatly to the overall sustainability of the cropping systems in which RC is used.

6. 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 maintained without compromising on profits. It is long established that legumes can offer farmers substantial economic returns and there has been international documentation of this; for example India (Ghosh, 2004), Kenya (Rao & Mathuva, 2000) Spain (Sanchez-Giron, Serrano, Hernanz, & Navarrete, 2004), and the USA (Biermacher et al., 2012). These returns are generally conceived of in direct ways such as the value of silage, reductions in fertilizer/fodder costs and yield increases (in comparison with conventional systems). The non-marketable impacts of improved soil fertility/structure and various ecosystem services (weed control and pollinator attraction etc) are much harder to quantify (Swinton, Lupi, Robertson, & Hamilton, 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 & Topp, 2002). Table 12 summarizes 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.

Doyle and Topp (2002) have highlighted the profitability of RC silages as an alternative to grass silage or bought-in feed, but this profitability may not also embrace the increased economic return from associated soil fertility or mitigation of soil erosion. Farmers practicing CA may require a significant percentage of aboveground biomass for green manure/mulch and it is difficult to determine if the value attributed to RC silage will also be realized for this purpose. Economic evaluations for RC in this regard generally compare yields and input costs of non-legume systems and RC systems under different management strategies, and then calculate the profitability from these differences. Katsvairo and Cox (2000) calculated the economic return for soybean-wheat/RC-corn rotations under three different tillage practices in New York State, USA. Table 13 summarizes the four-year average profitability of this rotation under differing tillage practices under both high and low chemical management.

Ridge tillage is a North American term for non-inversion tillage practices that makes use of crop residues for N and SOM and can therefore be considered a CA practice (Follett, 2001). The results of this study indicated that RC rotations using both high and low-input regimens under ridge tillage had a low economic return when compared to chisel mouldboard. However, it was also reported that the profits of the RC rotations did not account for the value of the wheat straw, which is typically traded between arable and dairy farmers in this area and can sell for up to $60 ha^{-1}. These additional gains would make RC rotations more profitable than conventional rotations, but would put competition pressure on residue-use which may reduce profits and impact negatively on the establishment and yield of the following crop. This trade-off illustrates the challenge of determining the economic return of cropping systems and, in addition, the results will be heavily influenced by the price of inputs and labour in that specific region and time. Other studies document the economic returns of RC systems under differing management strategies (Davis, Hill, Chase, Johanns, & Liebman, 2012; Meyer-Aurich et al., 2006; Stute & Posner, 1995), but again it is difficult to take these results and determine that RC rotations may be this profitable in other locations at different times. The profits also do not account for the long-term economic benefit of increased yield and input reduction through improved soil fertility, which may be a significant oversight given the focus of CA on soil conservation.

These difficulties in determining the economic efficacy of RC rotations in CA systems may be potential barriers to their adoption, and the economic basis involved can also be additionally impacted by external political factors. This review has highlighted that increasing availability of 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

Table 12. Value of RC silage in four European countries (Doyle & Topp, 2002).

Silage crop	UK	Germany	Sweden	Finland
	Value of silage produced (€/ha)			
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				
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				
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

Table 13. Comparison of average net profit from different crop rotations with high or low chemical input grown under different tillage strategies (Katsvairo & Cox, 2000).

Net Profit ($/ha)						
Tillage strategy	Chisel		Mouldboard		Ridge	
Chemical input	High	Low	High	Low	High	Low
Crop rotation system						
Continuous-Corn	13	1	49	−25	33	−27
Soybean-Corn	61	100	78	143	19	17
Soybean-Corn-Corn	54	46	84	115	36	26
Soybean-Wheat/Red Clover-Corn	24	26	69	108	5	6

soy and maize rotations, grown to produce fodder for industrial-livestock production (Weis, 2013) and biofuels (Lal, 2005). This is seen most strikingly in North America, where the political economy of grain/industrial-livestock production provides subsidies and greater profits to farmers willing to participate (Reganold, Papendick, & Parr, 1990). Conventional wisdom is that these agricultural models are probably more environmentally destructive and unsustainable, but there is less financial incentive for farmers to develop more sustainable practices more generally (unless they embrace a greener philosophy).

As this review has outlined, contributions to soil fertility and crop resilience made by RC can be difficult to predict. These predictions are even more challenging to translate into financial returns, but fortunately, the cost of business, as usual, is much easier to quantify. 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. In the UK alone it is estimated that the total financial cost of nitrogen leaching to health and the environment was £16m over a six year period (Pretty et al., 2000). In order for the economic sustainability of RC rotations to be fully realized therefore, farmers and governments will need to prioritize longer term sustainability more over short-term returns with respect to agricultural practices.

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