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Using perennial plant varieties for use as living mulch for winter cereals. A review

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Abstract

Both from the environmental and economical perspective, reducing the use of mineral nitrogen and herbicides is one of the future challenges in cereal production. Growing winter cereals on perennial legume living mulch such as white clover (*Trifolium repens* L.) or lucerne (*Medicago sativa* L.) is one of several options to reduce the need for mineral nitrogen fertilizer and herbicides in winter cereal production. Given the importance of winter cereals in the world, adopting this technique could greatly improve the sustainability of crop production. Through competition with the crop however, the living mulch can negatively affect cereal yield. Here, we (i) review how living mulch can be introduced in the system, (ii) synthesize potential advantages and disadvantages of that system, and (iii) explore different strategies to control the competition between the crop and living mulch. The major findings are that (i) competition between cereals and mulch can lead to significant yield reductions if not controlled properly and (ii) perennial legume varieties used as living mulch so far are varieties bred for forage production. We hypothesize that a dedicated breeding program might lead to living mulch varieties with a smaller impact on cereal yield compared to forage varieties, allowing to grow cereals with reduced nitrogen and herbicide inputs. We propose the main characteristics of an ideotype for such a perennial legume variety.

Keywords Wheat · Lucerne · White clover · Herbicides · Species mixtures · Nitrogen fertilization · Breeding · Ideotype

Contents

1. Introduction
2. Definitions and context
 - 2.1 In what agricultural systems direct seeding in living mulch could be useful
 - 2.2 Cereals in living mulch in practice
3. Potential advantages of sowing cereals into living mulch
 - 3.1 N fixation by the mulch and transfer to the crop
 - 3.2 Weed suppression
 - 3.3 Reduction in herbicide use
 - 3.4 Soil conservation
 - 3.5 Nitrogen losses
4. Intensity of the competition for light water and nitrogen between the living mulch and the crop
 - 4.1 Competition for light
 - 4.2 Competition for nitrogen
 - 4.3 Competition for water

5. Levers to control competition
 - 5.1 Herbicides
 - 5.2 Mechanical control of mulch
 - 5.3 Crop density and spatial distribution
 - 5.4 Crop species and varieties
 - 5.5 Mulch species and varieties
 6. Defining traits for living mulch varieties
 - 6.1 Prospects for breeding: a case study for lucerne
 - 6.2 Ideomix breeding
 - 6.3 Multi-species mulch
 7. Conclusion
- References

1 Introduction

There is an urgent need to decrease the negative environmental impacts of agriculture. In the European Union (EU), the farm to fork strategy, which is part of the European Green Deal, sets clear thresholds to decrease the impact of the food system (European Commission 2020). By 2030, the European Commission aims, among others,

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to decrease pesticide use by 50%, decrease nutrient losses by 50%, and decrease fertilizer use by 20%. Meanwhile, productive agricultural systems are still needed to improve European autonomy.

A greater reliance on legume crops and their biological nitrogen (N) fixation will play a key role in the reduction of mineral nitrogen fertilizer use. Different agricultural systems are calling for different legume-inclusive cropping systems (Ditzler et al. 2021). One of those systems is cropping cereals on living mulch mainly made of legumes (Fig. 1). The objective is that the living mulch would reduce weeds growth during the time interval between cereal harvest and sowing, and satisfy a proportion of cereal N demand. Although such an option was set more than 80 years ago, the use of living mulch in cereal production did not break through so far in none of the important cereal-producing regions of the world. An important drawback of growing winter cereals on living mulch is their mostly negative impact on yield due to competition between the living mulch and the crop, outweighing the potential positive impacts. Here, we review this potential yield loss and the potential advantages of living mulch for winter cereals in a quantitative way. Various options that were studied to reduce this competition and associate yield loss will be presented, analyzing how they currently work with respect to weed control and cereal N nutrition. Eventually, we came to the conclusion that the genetics of the living mulch was not considered an option to reduce competition so far: none of the species used as living mulch so far was ever bred for that purpose, ignoring the importance of cereal/companion species interactions on yield and N balance. We hypothesize that a dedicated breeding program might lead to living mulch varieties with a smaller impact on cereal yield compared to existing forage

varieties, allowing to grow cereals with reduced nitrogen and herbicide inputs.

The present review will focus on the use of perennial living legume mulch in winter cereal production. The use of living mulch in spring-sown crops like maize and soybean and vegetables has been reviewed elsewhere (Bhaskar et al. 2021; Hartwig and Ammon 2002). Despite the importance of winter cereals in countries such as Russia (producing 63 Mt of winter wheat in 2020/2021) and the USA (producing 50 Mt of wheat, mainly winter wheat in 2020/2021), almost all literature regarding winter cereals on living mulch came from European countries and especially from France (producing 30 Mt of wheat, mainly winter wheat in 2020/2021) (USDA 2022). The first part of this review focusses on the agronomy of producing winter cereals on living mulch. After defining the system and explaining its emergence, we (i) explore how the living mulch can be introduced in the cropping system, (ii) synthesize the potential advantages and disadvantages of that system, and (iii) explore different strategies to control the competition between the crop and living mulch.

In the second part of the paper, we hypothesize that breeding of dedicated varieties is needed to adopt the use of living legume mulch in arable production systems that are less dependent on mineral N and herbicides. Based on our experience in the breeding and ecophysiology of perennial forage plants, we propose an ideotype for perennial legume varieties dedicated for use as living mulch.

2 Definitions and context

Depending on the authors, the crops, and the cropping techniques, different definitions were used for “living mulch” (see Feil and Liedgens 2001 for an overview). A very general



Fig. 1 Cereals on legume living mulch. Left: winter wheat in lucerne living mulch (22/03/2022). Right: oats on lucerne living mulch (20/07/2021).

definition is given by Hartwig and Ammon (2002): “Living mulches are cover crops planted either before or with a main crop and maintained as a living ground cover throughout the growing season. If the living mulch is a perennial, it may be possible to maintain it from year to year without the need for reseeded.” In this review, we follow that definition; however, we consider living mulch as a perennial cover crop that is well established when the winter cereal is sown. Cover crops sown simultaneously with a cereal crop or undersown in an established cereal crop are considered relay intercrops (*sensu* Malézieux et al. 2009). For example, white clover undersown in early spring in organic winter wheat is called a relay intercrop (Amossé et al. 2013a). After the wheat harvest, the white clover sward can further develop. For a next winter cereal installed in this white clover sward, the clover sward is a living mulch.

To our knowledge, the first experiments with living mulch in arable crop production, reported in academic literature, were performed in the USA in the 1940s, in corn that was installed in existing clover swards. The initial driver for these experiments was soil erosion control. Later, the use of living mulch was studied in other spring-sown crops such as soy and vegetable crops with the aim to suppress disease and weeds. A good overview of these early experiments and more recent experiments regarding spring-sown crops and (living) mulch can be found in Hartwig and Ammon (2002), Feil and Liedgens (2001), Vincent-Caboud et al. (2019), and Bhaskar et al. (2021).

In Europe, where small grain cereals (soft wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), Durum wheat (*Triticum turgidum* L. var. *durum*), triticale (\times *Triticosecale* Wittmack), and rye (*Secale cereale* L.)) are the dominant arable crops (Eurostat 2021), the first published experiments to grow winter cereals in legume living mulch date from the 1990s (Table 1). Both in the organic as in the conventional context, research efforts regarding winter cereals sown in living mulch were increased in the first decade of the twenty-first century (Table 1), but so far this did not lead to a wide acceptance of the technique in practice. Although no academic scientific studies were published in the last 10 years, non-academic research continued by technical institutes and farmers. Especially in France, the technical institute Arvalis - Institut du végétal (www.arvalis.fr) and farmers continue to optimize the use of living mulch in cereal production (see for example Bodoville 2020).

2.1 In what agricultural systems direct seeding in living mulch could be useful

In the areas specialized in arable production in Europe, rotations are dominated by small grain cereals (wheat, barley, rye, oats), maize (grain and forage), and oilseed crops (oilseed rape and sunflower); together these crops represent

>90% of the EU arable land. By 2030, the European Union (EU 28, including UK) is expected to have an agricultural area of 174 Mha of which 55.6 Mha cereals (small grain cereals and grain maize) and 11.4 Mha oilseeds and 2.4 Mha grain legumes. Soft wheat (both winter and spring) dominates cereal production: the area is expected to increase to 23.8 Mha by 2030 (European Commission 2019). Actually, in the European Union (EU27, ex UK), France is the greatest cereal (small grain cereals and grain maize) producer with an annual production 71.2 Mt or 23.8% of all cereals produced in the EU in 2019, before Germany (14.8%) and Romania (10.2%) (Eurostat 2021). In these arable production regions of the EU, the rotations are mostly very short and cereal dominated. On the French arable land for example, dominant rotations were cereals–oilseed crop (28% of the arable land), cereals–maize (18%), and cereals–oilseed crop–maize (10%) according to the agricultural survey of 2017 (Agreste 2020). In such regions, there is a huge potential for adoption of living mulch adapted to cereal-based rotations. Statistics that quantify the surface of cereals actually grown on living mulch are, as far as we know, not available in Europe nor for other important cereal production regions. In Brittany, France, the use of living mulch is principally found in the following rotations: winter oilseed rape undersown white clover or lucerne–winter wheat on living mulch–winter wheat or winter barley on living mulch (Turlin 2016).

2.2 Cereals in living mulch in practice

Different techniques can be used to establish legumes as living mulch in a cereal-based rotation. Generally, the perennial legumes are sown in spring. Either as sole crop (Carof et al. 2007a), but mostly undersown in a companion crop like spring barley (Bergkvist 2003a), spring oat (Thorsted et al. 2006a), spring wheat (De Notaris et al. 2019), sunflower (Bodoville 2020), winter oilseed rape (Turlin 2016), or relay intercropped in winter wheat (Amossé et al. 2013a). This last option can be successful in organic agriculture, but in conventional winter cereals this technique is not adapted as wheat fertilized with mineral N is too competitive to allow the proper establishment of the legume. Sowing at the end of the summer (August) as a sole crop is another possibility to install perennial legumes in temperate maritime climates, but this results in an establishing living mulch sward that might be damaged upon winter cereal sowing in October–November (Shili-Touzi 2009).

Mostly, the perennial legumes are broadcasted or sown in rows with a conventional drill at rows spaced between 0.15 and 0.2 m (Hiltbrunner et al. 2007b), shortly before or after the companion crop (spring barley, sunflower, ...) is sown. Bodoville (2020) seeded lucerne in rows every 0.3 m between sunflower rows with an inter-row distance of 0.6 m using an RTK-GPS-guided precision drill. The next season, winter wheat was sown in between the living mulch rows.

Table 1 Overview of studies regarding cereals in living legume mulches.

Reference	Crop	Living mulches	Tested treatments to reduce competition	Age of mulch at cereal sowing	Annual N inputs	Average cereal yields
White and Scott (1991) (USA)	Wheat Rye	None <i>Medicago sativa</i> <i>Lotus corniculata</i> <i>Coronilla varia</i> <i>Trifolium repens</i> <i>Trifolium pratense</i>	None	3 months in year 1 15 months in year 2	0–56 kg N ha ⁻¹	Without mulch: 2.1 Mg ha ⁻¹ With mulch: 1.5 Mg ha ⁻¹
Jones and Clements (1993) (UK)	Wheat	<i>Trifolium repens</i>	Wheat sowing time Wheat sowing density	14 months in year 1 26 months in year 2	0–100 kg N ha ⁻¹	With mulch: 4.8 Mg ha ⁻¹
Thorsted et al. (2006a) (DK)	Wheat	None <i>Trifolium repens</i>	Width rototiller strips Wheat sowing density Wheat spatial arrangement	6 months in year 1 6 months in year 2	70 kg N ha ⁻¹	Without mulch: 4.5 Mg ha ⁻¹ With mulch: 3.7 Mg ha ⁻¹
Thorsted et al. (2006b) (DK)	Wheat	None <i>Trifolium repens</i>	Mechanical control of clover	6 months in year 1 6 months in year 2	70 kg N ha ⁻¹	Without mulch: 5.1 Mg ha ⁻¹ With mulch: 4.9 Mg ha ⁻¹
Bergkvist (2003a) (SV)	Wheat	None <i>Trifolium repens</i>	Herbicides	6 months in year 1 18 months in year 2	0–120 kg N ha ⁻¹	Without mulch: circa 7 Mg ha ⁻¹ With mulch: circa 6.0 Mg ha ⁻¹
Bergkvist (2003b) (SV)	Wheat	None <i>Trifolium repens</i>	Clover variety Wheat sowing density	6 months in year 1 18 months in year 2 30 months in year 3	60 kg N ha ⁻¹	Without mulch: 3.3 Mg ha ⁻¹ With mulch: 3.1 Mg ha ⁻¹
Carof et al. (2007a, b) (F)	Wheat	None <i>Lotus corniculatus</i> <i>Medicago lupulina</i> <i>Medicago sativa</i> <i>Trifolium repens</i>	None	8 months in year 1 20 months in year 2 32 months in year 3	> 200 kg N ha ⁻¹	Without mulch: circa 7 Mg ha ⁻¹ With mulch: circa 4 Mg ha ⁻¹
Hiltbrunner et al. (2007a, b) (CH)	Wheat	None <i>Lotus corniculatus</i> <i>Medicago truncatula</i> <i>Trifolium repens</i> <i>Trifolium subterraneum</i>	None	2 months in site 1 2 months in site 2	0–71 kg N ha ⁻¹	Without mulch: 3.8 Mg ha ⁻¹ With mulch: 0.8 Mg ha ⁻¹
Hiltbrunner et al. (2007c) (CH)	Wheat	None <i>Trifolium repens</i>	Wheat sowing density	2 months in year 1 site 1 14 months in year 2 site 1 3 months in year 1 site 2	60–95 kg N ha ⁻¹	Without mulch: 4.1 Mg ha ⁻¹ With mulch: 2.9 Mg ha ⁻¹
Hiltbrunner and Liedgens (2008) (CH)	Wheat	<i>Trifolium repens</i>	Wheat varieties	2 months in year 1 site 1 14 months in year 2 site 1 3 months in year 1 site 2	60–95 kg N ha ⁻¹	With mulch: 2.6 Mg ha ⁻¹
Humphries et al. (2004) (AU)	Wheat	<i>Medicago sativa</i>	Lucerne variety	7 months in both sites in year 1 14 months in both sites in year 2	0–22 kg N ha ⁻¹	Without mulch: 2.7 Mg ha ⁻¹ With mulch: 1.7 Mg ha ⁻¹
Shili-Touzi (2009) (F)	Wheat	None <i>Medicago sativa</i>	Herbicides	3 months in year 1 1.5 months in year 2	210 kg N ha ⁻¹	Without mulch: 8.2 Mg ha ⁻¹ With mulch: 1.6 Mg ha ⁻¹

In autumn (October–November), winter cereals are sown into these newly installed living mulch covers. This is done mostly with a minimal impact on the living mulch using a no-till seed drill (Carof et al. 2007a; Bergkvist 2003a; Hiltbrunner et al. 2007a). Another option is to cultivate the living mulch in narrow bands (for example, 0.09 m wide bands every 0.25 m) using a rotary cultivator and to sow the cereals in the cultivated strips (Thorsted et al. 2006a). Once the crop is installed in the living mulch, different techniques can be used throughout the growing season to control the competition between crop and living mulch (see Section 5).

In the next summer, after the harvest of the cereal, the living mulch serves a cover crop until a next cereal crop is installed. In the absence of competition for light from the cereal, the mulch can quickly develop as soon as the cereal crop is harvested, provided water is available. In this period, mulching the cover (cutting or chopping the cover, the cut or chopped material remains on the field and decomposes) can control or slow down the development of perennial weeds like thistles *Cirsium arvense* Scop. (Favrelière et al. 2020). Cutting and harvesting the mulch to feed cattle or a biogas plant is another option, but exports nutrients unless the digestate or manure is brought back to the field. Also before or shortly after the sowing of the cereal crop, it is recommended to cut/mow and mulch or to graze the living mulch rather short to eliminate competition for light between the living mulch and the emerging crop (Jones and Clements 1993).

A range of perennial legume species, all known as forage species, is used for living mulch (Table 1). The varieties used for installation of legume living mulch are varieties bred for forage production. So far, varieties specifically bred for use as living mulch are lacking, which is one of the barriers limiting the adoption of this technique in practice (see Section 5.5).

3 Potential advantages of sowing cereals into living mulch

Benefits associated with growing crops on legume living mulch generally cited include N transfer from the mulch to the crop and a decreased crop competition from weeds. In the period between cereal harvest and the sowing of the next cereal crop, the living mulch acts as a cover crop. N fixation, N uptake to prevent leaching, weed suppression, and soil conservation are the main roles of this cover crop. These benefits have been extensively reviewed before (Hartwig and Ammon 2002; Bhaskar et al. 2021). In the present paper, we will therefore focus on advantages for rotations based on winter cereals.

3.1 N fixation by the mulch and transfer to the crop

Mixtures of legumes and *Poacea* (grass, cereals) can yield more nitrogen than either of the monocultures, as shown for grass–clover mixtures (Nyfeler et al. 2011) and cereal–grain legume intercrops (Jensen et al. 2020). This mutual stimulation is based on two fundamental differences in N acquisition between both functional groups (Nyfeler et al. 2011): (i) legumes are mostly less efficient than cereals or grass to recover soil N and (ii) although legumes can turn atmospheric N₂ into plant N through symbiosis with N fixing bacteria, the uptake of soil N whenever it is available is more cost-effective in term of energy consumption than N₂ fixation. As a result, in mixtures of both, there will a non-proportional sharing of soil N sources. If the grass or cereal component dominates the canopy, it will take up most soil available N for which its higher biomass determines a higher N demand (Gastal and Lemaire 2002). Due to the scarcity of soil N created by the grass or cereal component, the legume component will rely on fixation of atmospheric N₂ to compensate its low share of soil N uptake. A second mechanism explaining the mutual stimulation between both functional groups is the direct transfer of N that is fixed by the legume to the grass or cereal component in the mixture through root exudates (i.e., release of organic and inorganic compounds from living plant roots) and/or mineralization of decaying legume root or shoot biomass. This direct transfer or rhizodeposition, however, was found to be negligible for cereal–grain legume intercrops (Jensen et al. 2020). In grass–legume mixtures, Louarn et al. (2015) found a difference among legume species for this direct transfer. The grass component in the mixtures could recover more fixed N from white clover (147 kg N ha⁻¹ over the 3 years) compared to lucerne (59 kg N ha⁻¹ over the 3 years). This finding can be explained by differences in root architecture between both species. White clover has finer roots with a lower C/N ratio that are more easily mineralized compared to the lucerne taproot. In addition, white clover releases more exudates than lucerne.

Although the same mechanisms that govern the N dynamics of grass–clover or cereal–grain legume mixtures will also apply to cereals grown on legume living mulch (Shili-Touzi 2009), there is a fundamental difference between both. In grass–legume or cereal–grain legume mixtures, the legume is harvested and has a direct economic value, whereas this is not the case in legume living mulch. Hence, every unit of soil N that is absorbed by the legume living mulch is not available for the crop of interest (cereal *in casu*). So the living mulch competes with the crop for N, unless the soil N uptake by the living mulch is compensated by a direct N transfer from the living mulch to the crop. The direct N transfer from legume living mulch to companion crops was scarcely quantified. In winter wheat grown in a first year

lucerne mulch, Shili-Touzi (2009) measured no transfer from legume N to fertilized winter wheat, but on the contrary, a clear N competition between the lucerne and the wheat. So during the cropping season, competition for N between the crop and the living mulch seems inevitable. This competition will be quantified in Section 4.

On the longer term, however, the N assimilated by the living mulch through the growing season will benefit to the next crop, either directly as soon as the vegetation starts decaying and is mineralized or indirectly if the living mulch is grazed by animals or chopped in the period between crop harvest and installation of the next crop. Shili-Touzi (2009) showed that the lucerne living mulch produced 2 Mg ha^{-1} aboveground biomass between the winter wheat harvest and the sowing of the next winter wheat. The N concentration and the proportion of N from fixation were not measured, but as 1 Mg lucerne aboveground dry matter production corresponds to $25\text{--}45 \text{ kg N}$ fixation (Jeuffroy et al. 2015), the N fixation associated with the 2 Mg ha^{-1} above ground biomass production between the harvest and the sowing of the next crop can be estimated as $50\text{--}90 \text{ kg N fixation ha}^{-1}$.

Chopping the legume living mulch to stimulate the transfer of N assimilated by the living mulch to the following crop prior to cereal sowing seems a good practice. In Scandinavian red clover leys, Dahlin et al. (2011) quantified the N recycling after mulching. They found that on average 83% of the N in mulched biomass was recovered in the regrowth and in the soil organic matter; the remaining 17% were, most likely, lost through volatilization. An integration of the living mulch practice into animal production or biogas production offers increased perspectives, but if badly managed, N losses in manure and digestate gaseous can also be important. The management allowing the most efficient transfer from the N assimilated by the living mulch to the crop should be further investigated. Whether cutting and harvesting the material to feed animals or a biogas plant and applying the manure or digestate to the crop in spring can lead to greater N fixation and smaller N losses compared to a scenario in which the living mulch is not harvested should be further explored.

Finally, after some cereal crop cycles, the living mulch is destroyed or ploughed down and the decomposing mulch root and shoot biomass will liberate N for the following crops. Owing to the smaller biomass production of a living mulch sward, covered by a crop during a great part of the growing season, compared to a legume ley, it is likely that ploughing down a living mulch will release smaller quantities of N for the following crops than those reported for legume leys (Cougnon et al. 2018). In addition, as mentioned by Lemaire et al. (2015), N is coupled to carbon and other minerals that contribute to limit nitrate leaching and nitrification.

3.2 Weed suppression

Legume companion plants decrease weed competition in crops according to a meta-analysis of Verret et al. (2017). In this meta-analysis, studies with three types of intercropping were considered: (i) living mulch (crop sown in established companion plant), (ii) relay intercropping (companion plant sown in established crop), and (iii) synchronized sowing (crop and companion plant sown simultaneously). Intercropping resulted in a lower weed biomass and a higher crop yield than non-weeded or weeded control treatments in respectively 52% and 36% of the studied experiments. For the studies regarding living mulch however, the weed-suppressing effect of the companion plants was not straightforward.

Negative impacts of weeds tend to increase with the age of the living mulch. In Carof et al. (2007a), studying winter wheat sown on different living grass and legume mulch species in three successive years receiving a great N input ($200 \text{ kg N ha}^{-1} \text{ year}^{-1}$) (Table 1), the legume living mulch decreased the weed biomass compared to a non-weeded pure wheat in the first year. From the second year on, several herbicides at low doses were applied in order to eliminate weeds and control the development of the mulch without killing it (see Section 5.1). In the second wheat crop sown on the living mulch however, weed biomass at wheat maturity was over 2 Mg ha^{-1} in white clover and black medic (*Medicago lupulina* L.) plots. In the third successive wheat crop, weed biomass was higher in all living mulch plots compared to the pure wheat plots. In all 3 years, wheat yield was lower on living mulch compared to pure wheat plots.

Also Bergkvist (2003a), studying winter wheat in white clover living mulch, reported increasing weed problems over time. He found practically no weeds in the first wheat crop, but the second wheat crop got heavily invaded by weeds, mainly *Apera spica-venti* P.B., *Elymus repens* Gould., *Lolium perenne* L., and *Papaver rhoeas* L. in one of the two locations.

The living mulch species influence the weed suppression. White and Scott (1991), studying winter wheat or rye sown on different living legume mulch species in two successive years with N fertilizations of 0 or 56 kg N ha^{-1} (Table 1), found an effect of the mulch species on the weed biomass from the second year of direct seeding of winter cereals in living mulch on. Averaged over both cereal species and N levels, weed biomass at cereal harvest reached 1.043 Mg ha^{-1} in the control treatment without living mulch whereas in the cereals on living mulch, the weed biomass was between 0.703 Mg ha^{-1} with crown vetch (*Coronilla varia* L.) mulch and 0.051 Mg ha^{-1} with lucerne mulch, none of the treatment being weed-controlled.

Similarly, Hiltbrunner et al. (2007a), studying organic winter wheat sown on four different living mulch species with or without manure application, found that the living mulch decreased both weed density and biomass. At the time of wheat anthesis, living mulch and weed biomass were measured on both sites and manure levels for all mulch treatments. Pearson's product-moment correlation was calculated between weed and living mulch biomass. Total weed biomass was negatively correlated with the mulch biomass ($r=-0.42$, $p<0.01$). The effect was significant for dicotyledonous weeds ($r=-0.41$, $p<0.01$) but not for monocotyledonous weeds.

Several mechanisms can explain the effect of living mulch on weeds, but aboveground competition for light is the most important (Petit et al. 2018). In winter wheat sown in lucerne living mulch, Shili-Touzi (2009) found that winter wheat and the associated lucerne living mulch absorbed 20% more photosynthetic active radiation (PAR) compared to pure wheat at the end of the winter. Although the difference between both decreased over time, the wheat–lucerne crop reached a maximal absorption (> 90%) in the wheat BBCH stage 32 (node 2 at least 2 cm above node 1), about 20 days earlier than the pure wheat crop. This restricted light access slows down the development of the weeds; moreover, the living mulch biomass changes the quality of the light (red/far-red ratio) affecting the germination of light-sensitive weed species (Petit et al. 2018, Westbrook et al. 2022).

The living mulch is beneficial for crop production if the competition between the crop and the living mulch is smaller than the competition between the crop and the weeds that were outcompeted by the living mulch. In contrast to (non-leguminous) weeds, legume living mulch fixes (a part) of the nitrogen needed to sustain its growth, leaving more N in the soil to sustain the crop growth, explaining the mainly beneficial effect of legume companion plants on both weed suppression and crop yield (Verret et al. 2017). Although this hypothesis sounds tempting, it denies the fact that legumes, whenever possible, prioritize to take up soil mineral N rather than to fix it (see Section 3.1). Moreover, the living mulch also competes with cereal for water and light (see Section 4).

Hence, living mulch, even if it is a legume, can compete with the crop, decreasing crop yields to an extent that is equal to the weeds that are suppressed by the living mulch. Westbrook et al. (2022) conclude that “It is relatively easy to suppress weeds with a living mulch but more difficult to do so without permitting excessive mulch-crop competition.”

3.3 Reduction in herbicide use

If living mulch can reduce weed presence on the crop level, this could be translated in a reduction of herbicide use on the agricultural system scale. Moreover, when implementing living mulch in a cropping system, herbicide use should be

reduced or adapted to guarantee the survival of the living mulch. On the other hand, supplementary applications can be necessary, especially when herbicides are used to control the competition between the crop and the living mulch (Section 5.1).

In the absence of studies allowing a direct comparison of herbicide use in cereal production with or without living mulch, it is not clear whether introducing legume living mulch in tight cereal-based crop rotations can lead to a reduced herbicide use. Adeux et al. (2019) used the herbicide treatment frequency index (HTFI), which is the sum of all herbicide treatments expressed as the proportion of the reference dose for a particular crop, to compare different cereal-based rotations. In a 17-year cropping system experiment, they have shown that a shift from a conventional arable cropping system with a 3-year crop rotation to a cropping system based on the principles of conservation agriculture, including use of living mulch in cereals and a 6-year crop rotation, allowed to decrease the HTFI by 9%. This reduced herbicide use came along with a yield decrease of 22%. Whereas the herbicide treatment frequency index (HTFI) of the conventional system was mainly represented by broad-spectrum herbicides, the conservation agriculture had a higher HTFI for anti-grass herbicides and glyphosate (Adeux et al. 2019; Cordeau et al. 2019). This comparison however is not very conclusive regarding the impact of living mulch on herbicide use as the difference between both systems cannot be attributed to the living mulch alone. Moreover, the HTFI does not take into account the environmental risk associated with the applied herbicides neither the risk for the operators applying the herbicides.

3.4 Soil conservation

The positive effects of leys, including living mulches, on soil erosion, soil structure, soil organic matter, etc. are well known (Martin et al. 2020). Specifically for living mulches in cereal production, Autret et al. (2016) quantified the effect of contrasting arable cropping systems on the soil organic matter content in a long-term cropping system experiment in Versailles, France. The studied cropping systems included, among others, (i) a conventional cropping system based on a pea–winter wheat–oilseed rape rotation combined with annual ploughing and (ii) a cropping system based on the principles of conservation agriculture (minimal soil tillage and maximal use of cover crops) with a pea–winter wheat–corn rotation, including red fescue (*Festuca rubra* L.) or lucerne living mulch in the winter wheat. During the period 1998–2014, the increase in soil organic carbon storage in the upper 0.3 m soil layer was significantly greater for the conservation agriculture–based cropping system (+ 625 kg C ha⁻¹ year⁻¹) compared to the conventional cropping system (+ 78 kg C ha⁻¹ year⁻¹). This increase in soil organic

carbon was mainly explained by the altered crop rotation and the use of living mulch and cover crops (80% of the increase) and to a lesser extent to the reduction of soil tillage (20% of the increase). This increasing soil organic matter can lead to a higher mineralization potential and increase the risk of N leaching over winter, unless fertilization and management are adapted accordingly (Constantin et al. 2012).

Direct seeding into living mulch has a clear positive effect on earthworm biomass and species diversity. Schmidt et al. (2001) compared earthworm biomass and species diversity in winter wheat monocrops and winter wheat sown in white clover mulch on four locations in the UK and Ireland during three to four successive growing seasons. The wheat with living clover mulch supported greater earthworm communities (137 g biomass m⁻²) than the wheat monocropping (36 g biomass m⁻²). Similar positive effect of direct seeding in white clover mulch on earthworm biomass and diversity was found in Brittany, France (Turlin 2016).

3.5 Nitrogen losses

The nitrogen losses of alternative arable cropping systems were quantified by Autret et al. (2019) in a long-term cropping system experiment in Versailles, France (temperate maritime climate). The average N surplus (N inputs–N exports) measured over the period 1998–2016 was greater in the conservation agriculture system (163 kg N ha⁻¹year⁻¹) compared to the conventional cropping system (63 kg N ha⁻¹year⁻¹), which was mainly explained by the important amount of N fixation by the lucerne living mulch/ley (149 kg N ha⁻¹year⁻¹). Despite the greater N surplus for the former, the continuous presence of living mulch or cover crops prevented greater N leaching: there was no significant difference in N leaching between both systems (21 kg N ha⁻¹year⁻¹). The N₂O emissions were monitored continuously between April 2014 and July 2017, using automatic chambers. Greater N₂O emissions (12 kg N ha⁻¹) were measured in the conservation agriculture system compared to the conventional cropping system (7 kg N ha⁻¹) over the whole monitoring period. The present study did not allow to attribute this greater N₂O emission in the conservation agriculture system either to the presence of the living mulch or to the reduced tillage.

Studies allowing a direct comparison of the reduction of nitrate leaching in the fallow period between successive autumn sown crops (e.g., winter oilseed rape–winter wheat; winter wheat–winter barley) (i) grown on living mulch versus (ii) grown without living mulch but followed by an annual cover crop (e.g., mustard *Sinapsis alba* L.) are missing. Fast-developing cover crops from the *Brassicaceae* (*Sinapsis alba* L., *Raphanus sativus* L.), sown after cereal harvest, can reduce the soil mineral N quantity, prone to

leaching, with 90 kg N ha⁻¹ before the start of the winter (Vos and van der Putten 1997). Sowing cover crops in the short interval between successive autumn crops, however, is rather uncommon in practice. Cereal volunteers and the weeds developing after cereal harvest can take up as much N as a sown cover crop in many cases (Macdonald et al. 2005). Owing to their well-developed root system at cereal harvest, living mulch has a great potential to reduce soil mineral N in the short time frame between two succeeding autumn sown crops. Despite their ability to fix N₂, legumes take up mineral N whenever available (Section 3.1).

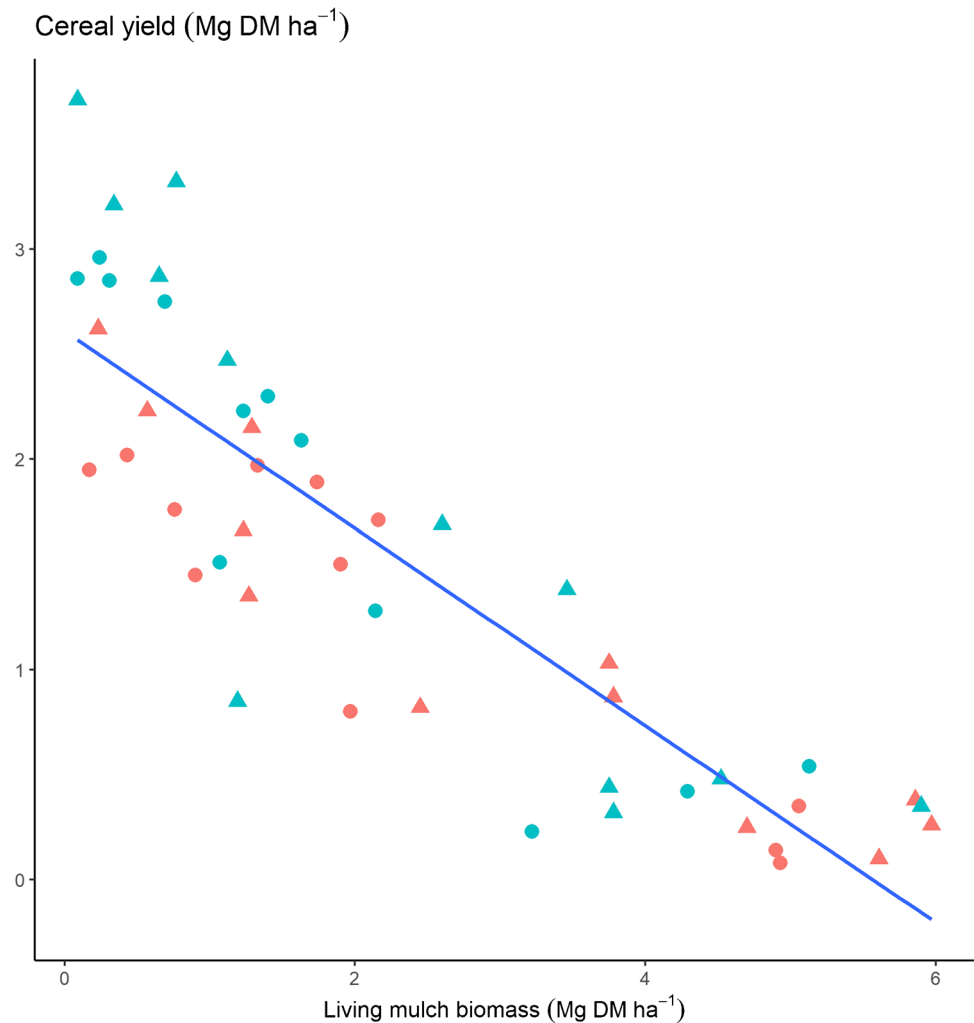
4 Intensity of the competition for light water and nitrogen between the living mulch and the crop

Living mulch competes with the crop for various resources. As soon as the competition is greater than the advantages delivered by the living mulch (see above), the presence of the mulch leads to yield losses, generally increasing with the living mulch biomass. The threshold of living mulch biomass at which this yield loss occurs depends on many factors like living mulch species (Carof et al. 2007a), resource availability (Shili-Touzi 2009), and yield potential of the crop (Amossé et al. 2013b). In the end, the economic balance is what really counts for the farmer. Modelling this economic optimum in the light of fluctuating prices of inputs (fuel, mineral nitrogen), outputs (cereal), and ecosystem services is outside the scope of this review.

If nothing is done to reduce the competition between crop and living mulch, the cereal yield losses can be very important. White and Scott (1991) studied winter wheat or winter rye sown in living legume mulch in two successive years with annual N fertilizations of either 0 or 56 kg N ha⁻¹. In this study, every kg ha⁻¹ of living mulch biomass measured at cereal harvest resulted in a grain yield loss of 0.57 kg ha⁻¹ of cereal (Fig. 2). Grain yield ranged between 3.69 Mg ha⁻¹ for winter wheat without living mulch receiving 56 kg N ha⁻¹ and 0.87 Mg ha⁻¹ for winter wheat sown on a red clover mulch.

Also in experiments with greater N fertilization (200 kg N ha⁻¹ year⁻¹) (Carof et al. 2007a), grain yield losses of between 19 and 81% relative to pure winter wheat were found with increasing living mulch and weed biomass. An in-depth study of the yield components of the wheat showed that plant density, tiller number per plant, and grain weight were rarely affected by living mulch. The yield loss associated with the living mulch was mainly explained by a reduction in the proportion of tillers that developed into ears and the number of grains per ear, which indicates that the crop–mulch competition was strongest from wheat stem elongation (BBCH stage 30) to the fecundation (BBCH stage

Fig. 2 Cereal yield as a function of living mulch biomass for winter wheat (\blacktriangle) or rye (\bullet) sown in living legume mulch in two successive years (1983–1984) with a yearly nitrogen fertilization of 0 kg/ha (red symbols) or 56 kg/ha (green symbols) and the linear trend line through the points ($y = 2.61 - 0.47x$, $p < 0.001$, $R^2 = 0.75$) (figure based on the data of White and Scott 1991).



61) (Carof et al. 2007a). Accordingly, Shili-Touzi (2009) found a wheat yield reduction of 80% due to living lucerne mulch compared to a pure wheat yielding 8.2 Mg ha^{-1} . A reduced ear density and number of grains per ear explained this yield reduction.

The cereal yield loss associated with living mulch is in most cases partially compensated by an increased grain protein concentration, which is an important quality parameter for wheat (Bergkvist 2003a; Thorsted et al. 2006a; Hiltbrunner et al. 2007b). For example in Thorsted et al. (2006a), white clover living mulch decreased winter wheat yield from 4.7 to 4.14 Mg ha^{-1} , but increased grain protein content (N concentration $\times 5.7$) from 8.0 to 9.3% . In the second year of their trial, living mulch decreased yield from 4.4 to 3.3 Mg ha^{-1} and increased grain protein concentration from 7.9 to 9.2% . Grain N yield was unaffected or slightly decreased by the presence of the living mulch. This indicates that this increased protein concentration associated with living mulch is rather explained by the lower cereal yield than by an improved N nutrition. The negative relationship between

cereal yield and protein concentration is known as the nitrogen dilution effect (Justes et al. 1994).

4.1 Competition for light

In studies with high N input ($>200 \text{ kg N ha}^{-1}$) (Carof et al. 2007b; Shili-Touzi 2009), competition for light between cereal and living mulch had the greatest impact on crop yield. Although wheat was taller than the living mulch throughout the growing season, Carof et al. (2007b) found important competition for light in winter wheat sown on living mulch. In the third successive wheat crop grown on living mulch, they made a radiative balance for winter wheat on legume living mulch. Measurement of the incident and reflected photosynthetic active radiation (PAR) above the whole crop and the PAR transmitted through the upper crop layer, dominated by the wheat, and the lower, mixed crop layer, where both wheat and living mulch occurred, allowed them to calculate the PAR intercepted by wheat and the living mulch. For wheat sown on lucerne, for example, in the

canopy layer where both wheat and living mulch occurred, wheat intercepted $108 \text{ mmol m}^{-2} \text{ s}^{-1}$ and lucerne $64 \text{ mmol m}^{-2} \text{ s}^{-1}$ averaged over the 65 days following the wheat BBCH stage 29 (end of tillering stage).

4.2 Competition for nitrogen

The competition for light induces the competition for nitrogen, because the N demand directly depends on the crop biomass, itself largely depending on the intercepted radiation. In order to separate competitive relationships for light and nitrogen, the nitrogen nutrition index (NNI) of the winter wheat can be calculated (according to Justes et al. 1994). This NNI accounts for the negative relationship between plant biomass and N concentration. NNI equal or higher than 1 indicates that N is not limiting plant growth. A lower NNI of wheat on living mulch compared to wheat without living mulch is an indication for competition for N between the crop and the mulch. Both in Carof et al. (2007b) and Shili-Touzi (2009), competition for N between wheat and living mulch was mostly absent at the BBCH stage 29 (end of tillering), but strong competition occurred at the BBCH stage 61 (beginning of flowering), despite the rather high N inputs in both studies. In Shili-Touzi for example, NNI decreased from 0.9 at the end of tillering to 0.48 at flowering, whereas that of pure wheat remained around 0.9 throughout the whole period. In Carof et al. (2007b), in the first wheat crop on living mulch, the NNI of wheat on living mulch at wheat flowering was significantly lower compared to the NNI of pure wheat. In the second and third successive wheat crops on living mulches however, competition for N between wheat and living mulch rarely occurred, except for white clover and black medic treatments that got invaded by weeds from the second year on. It is not clear if this smaller effect of the living mulch on the NNI of the wheat can be explained by an increased N transfer of legume N to the wheat as from the second year on, N fertilization for the wheat on living mulch was increased and herbicides were applied to control weeds and living mulch in this study.

Despite the ability to fix N, the leguminous living mulches take up mineral N from soil when available and can compete with the cereal for the applied mineral fertilizer (Section 3.1). Shili-Touzi (2009) measured the percentage of lucerne N derived from N fixation in winter wheat on living lucerne mulch using the natural abundance of ^{15}N . Winter wheat was amended with $210 \text{ kg N ha}^{-1} \text{ year}^{-1}$, split in three fractions. Before the first mineral N fertilization of the wheat (at BBCH stage 31), the proportion of N coming from atmosphere was equal in lucerne living mulch and in a pure, unfertilized lucerne sward (83%). At wheat harvest, the proportion of N from atmosphere in lucerne living mulch

decreased to 17%, whereas, in the pure, unfertilized lucerne, it was still 61%. This is in contrast with what is found in mixed intercrop systems of annual grain legumes and cereal crops (e.g., barley–pea sown simultaneously), where even in fertilized crops, legumes fix the majority of their N. Cereals showed to be more competitive than the grain legumes in extracting soil mineral N through a faster root growth of the former compared to the later. The lead of the cereals rooting depth allows the cereals to extract an important part of the available mineral soil N at a given soil depth before the grain legumes can access this N (Corre-Hellou et al. 2007). In cereals sown in living mulch, on the other hand, the advanced growth stage and rooting depth of the living mulch at the moment the cereal is sown explains why the legumes can strongly compete with the cereals for soil mineral N in that system.

4.3 Competition for water

Winter crops, at least in Europe, rarely face severe droughts. However, Brisson et al. (2010) suggested that part of the wheat yield stagnation observed in France and other countries since the late 1990s might be due to the more frequent occurrence of water deficits in spring. Indeed, water was not a limiting factor for wheat yield in Carof et al. (2007b). Similarly, Shili-Touzi (2009) concluded that water was not limiting wheat yield, despite the smaller plant available water content measured in the 0–0.9 m soil profile of wheat on living lucerne mulch versus pure wheat. However, as the legume living mulch transpires more water than a bare soil as illustrated for maize grown on legume living mulch (Sanders et al. 2018), the presence of the living mulch is likely to reduce the wheat yield potential in dry springs or on soils with a low water holding capacity and dry years. Despite a somewhat different root system architecture, the perennial legume and cereal extract water from the same horizons. The water demand is directly driven by the solar absorption of the canopy, again and as for nitrogen, the competition for light determines the competition for water. In years of low rainfalls, and on shallow soils indeed, if the older legume crop exhibits a larger root system, the risk for the living mulch to bring about more severe water deficits cannot be ignored. Although not of a first order, root traits might be important to consider when choosing the right legume cultivars to be associated to the winter cereal. Finally, as already observed under dry spring conditions (Klem et al. 2018), water deficits might also increase the competition for nitrogen, due to the impact of water deficits on nitrogen nutrition (Gonzalez-Dugo et al. 2010). Drought can limit N transport to the cereal rhizosphere and local N availability (Durand et al. 2010) and strongly alter N fixation of the legume (Marino et al. 2007).

5 Levers to control competition

Several strategies were tested to decrease the crop–mulch competition in winter cereals grain on legume living mulch (Table 1).

5.1 Herbicides

Herbicides used to kill dicots in cereal crops, when applied at reduced doses, can decrease the crop–mulch competition without jeopardizing the development of the mulch after crop harvest.

Bergkvist (2003a) applied the herbicide tribenuron-methyl (1.5 g ha^{-1}) in winter wheat with white clover living mulch in early spring with the aim to reduce white clover biomass. The effect of this herbicide treatment was tested in two successive years on the same clover swards on two locations. In the first year of the experiment, depending on the location, the herbicide application increased the grain yield by (averaged over the different N fertilization levels) 1.4 and 0.4 Mg ha^{-1} compared to wheat in untreated living mulch. In the second wheat crop, there was no effect of the herbicide application. Summed over both years, there was a positive effect (+ 8%) for grain yield in one of both locations for the wheat on herbicide-treated white clover mulch compared to a pure wheat crop. Clover presence in autumn was not affected by the spring herbicide treatment.

Shili-Touzi (2009) studied the effect of 2,4-D applications, at $\frac{1}{4}$ of the recommended dose for weed control, on the functioning of wheat sown in living lucerne mulch. The experiment was repeated 2 years. In the first year, the herbicide applied in the wheat BBCH stage 32 (node 2 at least 2 cm above node 1) at a dose of $210 \text{ g 2,4-D ha}^{-1}$, stabilized the growth of the lucerne living mulch biomass at 2 Mg ha^{-1} whereas the uncontrolled living mulch biomass increased till 4 Mg ha^{-1} . Despite this successful control of the lucerne biomass, the herbicide application could not prevent an important wheat yield loss associated to the living mulch presence. At harvest, the aboveground wheat biomass yield of the wheat grown on the chemically controlled lucerne mulch was only 50% of the yield of pure wheat ($17.4 \text{ Mg DM ha}^{-1}$). After wheat harvest, the regrowth of living mulch with and without herbicide treatment was similar. In a next growing season, the effect of the time of application was tested: 2,4-D was either applied in the BBCH stage 30 (beginning of stem elongation) or in the BBCH stage 32. The earlier herbicide treatment had a greater effect (51% lucerne biomass decrease compared to the untreated living mulch) than the later treatment (37% biomass decrease). Again, despite the lucerne biomass reduction, yield losses compared to the pure wheat remained important: 33% and 42% compared

to the pure wheat for the early and late herbicide treatment, respectively. Moreover, the herbicide application to reduce lucerne competition led to an increase of the weed biomass, especially of weeds that were tolerant to the herbicide (e.g. ryegrass) or weeds that were protected by the lucerne canopy due to their smaller size. Nevertheless, the weed biomass at wheat harvest remained below 1 Mg ha^{-1} for all treatments. The herbicide treatment of the living mulch did not increase the nitrogen nutrition index of the wheat compared to the wheat on the untreated mulch.

Based on a network of field trials in France, 50 comparisons were made of winter cereal yield sown either with or without living legume mulch, but with chemical weed and living mulch control (Labreuche et al. 2017). Living mulch biomasses below 1 Mg ha^{-1} at BBCH stage 60 (flowering of the wheat) had a neutral or positive effect on wheat yield compared to pure wheat. Beyond that threshold, a strong negative effect of the living mulch on wheat yield occurred. By adapting the herbicides, the application stage, and the doses, herbicide schemes can be found that eliminate weeds and reduce competition from living mulch. For French growing conditions for example, the French technical institute Arvalis proposes different herbicide schemes for controlling weeds and regulating the growth of lucerne or white clover living mulches in winter cereals (Arvalis 2020).

From these studies, it seems that growing cereals in living mulch can result in a shift of herbicide use: reduced doses but increased treatment frequencies. The use of chemical control of living mulch is therefore not necessarily in contradiction with the targets set in the European Green Deal (European Commission, 2020) regarding reduction of pesticide use: small doses of herbicide might be sufficient to reduce competition of both weeds and living mulch to an acceptable degree. On the other hand, living mulch excludes the use of mechanical weed control techniques which might be a more predictable and robust alternative for herbicides in arable crop production. Hoeing and harrowing work well in winter cereals with a limited impact on crop yield (Naruhn et al. 2021).

As discussed in Section 3.2, it is not clear from the environmental point of view, whether growing cereals with living mulch, that is controlled using herbicides, results in an environmental gain compared to pure wheat crops with chemical or mechanical weed control.

5.2 Mechanical control of mulch

Partial destruction of the living mulch at cereal sowing such as in strip tillage can be used to reduce the early competition for light between crop and mulch. Thorsted et al. (2006a) studied the effect of the width of rototilled strips at sowing

of winter wheat in white clover mulch on yield and quality. Rototilled strip width was either 0.07 m or 0.14 m for a row distance of 0.25 m. In one out of two years, the yield of the wheat sown in the 0.14 m strips (4.34 Mg ha^{-1}) was significantly higher compared to the wheat in the 0.07 m strips (3.82 Mg ha^{-1}). Another, more drastic, option to decrease early crop–mulch competition is cultivating the whole living mulch surface using a rigid tine cultivator or a disc cultivator just before cereal sowing (Bergkvist 2003a). Jones and Clements (1993) suggest autumn grazing of the living mulch to decrease the competition during the cereal establishment phase.

Once the crop is established, mechanical control of the living mulch in the crop can further reduce the crop–mulch competition. Thorsted et al. (2006b) studied the effect of one to three interventions with a weed brusher in white clover living mulch in different cereal developmental stages. These weed brushes tear off the plant material at soil level in 0.11 m wide strips in winter wheat with an inter-row distance of 0.25 m. The weed brushings took place between the BBCH stages 23 (three tillers visible) and 39 (flag leaf stage). The greatest effect of a single brushing was obtained in the stem elongation phase of the wheat with grain dry matter yield increases of 0.98 and 1.11 Mg ha^{-1} in the first and second year of the experiment. Wheat that was brushed twice or more had a similar or greater yield per hectare than pure wheat. The cutting and mulching of the clover aboveground biomass by the weed brushers clearly increased the N transfer from clover to wheat compared to wheat with untreated living mulch.

Likewise, experiments were performed in organic winter wheat in France with a specially designed mulch mower that allows to chop lucerne living mulch in between the wheat rows, sown with a row distance of 0.3 m (Bodoville 2020). Increasing row distance to values superior to 0.2 m in small grain cereal however often results in yield decreases (Melander et al. 2005).

Although these experiments proved that the mechanical control of the living mulch in the crop succeed to reduce competition between crop and living mulch, the impact of these interventions on the agricultural system scale was not studied. Repeated mechanical interventions have a high labor and energy cost. Moreover, the additional traffic on field associated with the mechanical control methods can result in increased soil compaction and a long-term reduction of yield. With the available literature, it is not clear whether mechanical control of living mulch in winter cereals is economically viable and which effect they have on the carbon footprint of the produced cereal. A complete balance of the greenhouse gas emissions, using a life cycle analysis approach (see for example Plaza-Bonilla et al. 2018 for different cropping systems), would be necessary to evaluate the environmental impact of the introduction of these techniques.

5.3 Crop density and spatial distribution

Increasing uniformity of crop spatial distribution and crop density can increase its competitive ability to weeds (Weiner et al. 2001). Likewise, crop–living mulch competition is also influenced by the crop density and inter-row distance.

Increasing seed density resulted in linear increases of plant, tiller, and ear density and eventually in a significant yield increase in trials where winter wheat was sown in white clover mulch at densities of 300, 450, or 600 viable grains m^{-2} with an inter-row spacing of 0.375 m (Hiltbrunner et al. 2007c). The optimal seeding density is strongly dependent on many environmental factors such as for example inter-row distance and living mulch species. For winter wheat sown at an inter-row spacing of 0.22 m in white clover mulch, Jones and Clements (1993) found the highest yields for a sowing density of 250 plants m^{-2} .

The role of the crop spatial arrangement was studied by Thorsted et al. (2006a). Winter wheat sown in white clover living mulch at a density of 400 kernels m^{-2} in 0.14 m wide rototilled strips spaced by 0.25 m, yielded up to 17% more when sown in two rows, and spaced by 6 cm rather than in one row within each rototilled strip (Thorsted et al. 2006a).

The quantitative effect of these measures however is relatively small compared to the effect of the mechanical or chemical control measures. Indeed, increased crop density and spatial uniformity can be expected to have large effects on weed or *in casu* living mulch growth when the crop has a significant initial size advantage over the weeds when competition begins (Weiner et al. 2001). This condition is not met in the case where the living mulch has a greater initial size than the crop, limiting the potential of its suppression through increased crop density or spatial uniformity.

Decreasing the sowing density of the living mulch does not necessarily have a positive effect on crop yield (Bhaskar et al. 2021). Especially for stoloniferous species such as white clover, sowing density does not reflect the density of the growing points in the resulting sward. If sowing density of the mulch is too low, ground cover by the living mulch will be incomplete and weeds might fill the gaps. Finally, the choice of the best combination between the winter cereal cultivar and the legume plant material is critical for optimizing the management of the living mulch.

5.4 Crop species and varieties

Between and within cereal species differences for their competitive ability against weeds or intercropped plants are well known (Blaser et al. 2011; Drews et al. 2009). White and Scott (1991) found that winter rye, owing to its taller growth, suffered less from living mulch competition than winter wheat. Compared to pure wheat and rye crops,

yielding 2.91 Mg ha⁻¹ and 2.42 Mg ha⁻¹, respectively, living mulch decreased the grain yield by 5% in winter rye compared to 15% in winter wheat in the first year of the trial. In the second year, when the legume mulches were more competitive owing to their better establishment, this effect was exacerbated: pure wheat and rye crops yielded 1.77 Mg ha⁻¹ and 1.6 Mg ha⁻¹ respectively, but the living mulch decreased grain yields by 28% in winter rye and by 69% in winter wheat.

The yield of different winter wheat varieties with white clover living mulch was tested in Switzerland by Hiltbrunner et al. (2008). In their trials, the tallest wheat varieties generally had the highest yield. In the absence of data from plots without living mulch, it is difficult to evaluate whether the greater yield of these taller varieties is due to their greater competitive ability against the living mulch or rather due to an intrinsically higher yield potential.

In a study comparing the weed-suppressing ability of winter wheat varieties, Drews et al. (2009) found that the more competitive varieties had a greater ground cover and thus intercepted more light through a combination of planophile leaf inclination, higher leaf area index and higher crop height. The greater the inter-row distance, the greater the advantage of planophile leaves for the suppression of weeds (Drews et al. 2009). Hence, the competitive ability of wheat varieties against weeds or living mulch is determined by a combination of traits and depends on environmental factors like inter-row distance. Nevertheless, varieties with a good tillering ability, tall stature, and early ground cover offer the best perspectives to be more competitive against living mulch.

5.5 Mulch species and varieties

In winter cereals, lucerne and white clover are the forage species that were most often studied as living mulch for cereals (Table 1). As both are important forage species, many varieties are available in both species allowing selecting varieties with traits that are compatible with the intended use and the geographical area. Soil and climate of a specific site limit the species choice. Lucerne for example requires a higher soil pH (6.5–7.5) than white clover pH (6.0–6.5) for successful establishment, but it supports drier and hotter summer conditions compared to clover (Annicchiarico et al. 2015).

Birdsfoot trefoil (*Lotus corniculatus* L.) has a lower yield potential under most circumstances than white clover or lucerne (Schneider et al. 2015), and can be therefore less competitive for the cereal. Nevertheless, in some circumstances, it proved to be very competitive. Of the six living mulch species compared in the study of White and Scott (1991), birdsfoot trefoil produced the lowest biomass (0.4 Mg ha⁻¹ at cereal harvest) in the first year of the trial,

whereas in the second year, it was the most productive species, together with lucerne (5 Mg ha⁻¹). In Carof et al. (2007a), birdsfoot trefoil living mulch resulted in lower wheat yield losses compared to lucerne or white clover. Winter wheat yield on birdsfoot trefoil living mulch was equal to wheat without living mulch in one out of the three trial years, whereas the wheat yield on lucerne and white clover living mulch was significantly lower compared to the pure wheat in the 3 years of the experiment. Legume species that are less competitive towards the crop might lack persistence and do not suppress weeds sufficiently.

Although not perennial, subterranean clover (*Trifolium subterraneum* L.) and black medic (*Medicago lupulina* L.) constitute canopies, which clearly can be considered living mulches. Owing to its particular life cycle, the winter annual legume subterranean clover has gained interest for use as living mulch for summer crops (soy, maize, vegetables) (Ilnicki and Enache 1992) but also for winter cereals (Radicetti et al. 2018). The plant initiates growth in late summer or early fall, grows vegetatively until early winter, becomes dormant in winter, and resumes vegetative growth the following spring. Later in the spring the plant flowers, seeds are produced and mature in a burr at or below the soil surface, then the plant senesces and dies (mid-June), resulting in a dense mulch of dead subterranean clover. Beginning in late July until mid-August, the seeds produced in the spring begin to germinate and by early September, a cover of living mulch is again produced (Ilnicki and Enache 1992). The Mediterranean origin of this species limits its use to regions with mild winters; winter in the Atlantic-North part of Europe proved to be too cold for survival of subterranean clover (Radicetti et al. 2018). However, ecotypes with a frost resistance that is sufficient to survive winter in South-Germany were found (Baresel et al. 2018), which opens perspectives to use this species more North. In contrast with perennial legumes species such as lucerne or white clover, there is no living subterranean clover vegetation in summer, limiting the biomass production and N fixation in the post-harvest period until the installation of the next cereal crop. Seed production for this species is difficult: as the seed are buried in the ground, special equipment is needed for seed harvest. Currently, only in Australia seed is produced on a commercial scale (Baresel et al. 2018). Hence, seed is relatively expensive, which could be major constraint to use subterranean clover mulch in practice.

Exploiting the genetic variance within living mulch species used in winter cereals to reduce competition is an option that was only scarcely studied so far. Bergkvist (2003b) studied the effect of white clover varieties used as living mulch on yield of winter wheat and winter oilseed rape in relation to the white clover traits. Small leaved white clover varieties produced less biomass in spring compared to medium size leaved varieties; as a result, winter oilseed rape sown in

a mulch of the former had a greater yield compared to the latter. For winter wheat, no clear effects of the compared clover varieties were found. Also Turlin (2016) recommended to use small leaved white clover varieties for living mulch in winter wheat. The effect on winter wheat yield sown on lucerne living mulch with contrasting winter activity classes was studied by Humphries et al. (2004) in Southern Australia. Yield penalty was smaller on the winter dormant lucerne mulch than on the winter active mulch (Table 1).

The great intra-specific variation for traits like plant architecture, biomass production, and flowering date (Julier et al. 1995) in lucerne suggests that lucerne varieties or populations could be found that are more suited to serve as living mulch than the forage-type varieties used in previous studies (Carof et al. 2007a; Shili-Touzi 2009).

6 Defining traits for living mulch varieties

Although scarcely studied, the use of specific genotypes, populations, or varieties of living mulch could offer great opportunities. Dedicated breeding programs might lead to living mulch varieties with a smaller impact on cereal yield compared to living mulch made of forage varieties. The breeding effort and gap between the currently cropped varieties and desired ones cannot be underestimated.

An ideotype can be defined as a biological crop model based on a combination of morphological and physiological traits to meet the needs in terms of production for a given environment or production system (Desclaux et al. 2013). In the absence of any research on the topic, it is difficult to define a cereal ideotype for growth on living mulch. Moreover, growing cereals on living mulch could be interesting in a wide range of cropping systems (organic *versus* conventional), calling for different ideotypes. It is clear however that the cereal varieties should be able to cope with the competition of living mulch and weeds (Section 3.2). Hence, the cereal ideotype defined for organic wheat production (Drews et al. 2009, Mason et al. 2007, Lammerts van Bueren et al. 2011) can serve as a model for production on living mulch. Cereal traits associated with competitive ability against weeds include good soil cover through planophile leaf inclination or high leaf area index, great plant height, and great tillering capacity.

Similarly, it is difficult to define a living mulch ideotype in the absence of research on functional traits useful for living mulch and given the multitude of cropping systems in which living mulch could be interesting. Many varieties are available for perennial legume species such as lucerne and white and red clover, but forage production and yield stability over cuts and years are the main trait that drives the breeding of new varieties (Annicchiarico et al. 2015). Owing to the negative effect of living mulch biomass production on

cereal yield potential, it is evident that a different perennial legume ideotype is needed for use as living mulch compared to forage production. Genetic traits must be defined dynamically, the time dimension being added to the 3D dimension. The morphogenesis of varieties of mulch and cereal species will need to be adapted in order to minimize the competitions for resources. The time in the crop cycle where the maximum demand of the cereal for light and nitrogen should be the time where the demand of the mulch species should be kept as low as possible.

Based on the literature research given above, the following traits should at least be taken into account in the breeding of dedicated perennial legumes for use as living mulch:

- Plant architecture dynamics: short prostrate legume types are clearly more suited compared to tall erect types for use as living mulch. Firstly, competition for light in the stage before grain filling was found to be the main reason for wheat yield decrease through living mulch in Carof et al. (2007b). To minimize competition for light between the cereal and the living mulch, the living mulch should be short. Secondly, at harvest, green biomass is unwanted in the harvested cereal as it will negatively affect the threshing in the combine harvester and the water concentration of the harvested grain. Living mulch height should therefore remain well below the ear height. Thirdly, in the period between cereal harvest and the sowing of the next crop, prostrate rhizomatous or stoloniferous types are better suited than erect types to cover the soil, to colonize the gaps in the living mulch made by sowing and harvesting machinery, and finally to suppress weeds. Whereas species such as white clover have a pronounced prostrate architecture, this is clearly not the case of the current lucerne forage varieties in living mulch, which can grow taller than winter wheat (Carof et al. 2007b). The wide genetic variation in lucerne however offers opportunities to select types adapted for use as living mulch (see below).
- Plant phenology: winter dormant types, which start their growth late in spring, allow the winter cereal, sown in the living mulch, to take a lead in the development to compete with the mulch. The critical period for competition in winter wheat, i.e., the period when weeds are likely to exert their greatest competitive effect on the crop, was found from tillering (BBCH 21) till the first node is 1 cm above the tillering node (BBCH 31) (Masson et al. 2021). Therefore winter dormant types, which start their growth late in spring are to be preferred. After the cereal harvest, summer and early autumn growth should be very vigorous, to allow a maximal biomass production, weed suppression, and N fixation in the period between two crops. Late autumn growth is unwanted to avoid competition once the winter cereal is sown.

- Disease resistance: excellent resistance against root pathogens will be needed if the aim is to keep the living mulch alive for several years in succeeding crop rotation cycles. To avoid problems with root disease in lucerne forage production, a 4-year break is recommended between two lucerne crops. In the rotations where living mulch could be integrated, this break between two succeeding living mulch is not an option, calling for types with an excellent disease and pest resistance.

Depending on soil and average climate conditions, as stated before, different species can be used as living mulch. Breeding prospects and methodologies for living mulch are exemplified in lucerne.

6.1 Prospects for breeding: a case study for lucerne

Within the *Medicago sativa* complex of species, a wide genetic variation is available. The genetic variation present in the present forage varieties is only a fraction of the variation present in the species. Most of the cultivated forage varieties belong to the subspecies *sativa* and are tetraploid, characterized by an erect growth habit. The *falcata* subspecies, which is scarcely used as forage crop, is characterized by a prostrate growth habit, strong winter dormancy, and winter hardiness. In both subspecies, diploid and tetraploid forms are present (Annicchiarico et al. 2015). Julier et al. (1995) characterized the morphology and biomass production of cultivated (varieties or landraces) and wild populations of the *M. sativa* complex, including diploids and tetraploids of subsp. *sativa* and subsp. *falcata*. The cultivated, tetraploid *sativa* populations were the tallest and the most productive of the studied populations (Table 2). There were also clear differences in the date of onset of growth and in the autumn growth. Populations with traits interesting for use as living mulch, such as lower plant height, reduced spring and autumn growth, were found in the *falcata* group but

also in wild tetraploid *sativa* material. Hence, for use as living mulch, varieties based on these latter types are expected to be more adapted. Differences in phenology of contrasting lucerne varieties are illustrated in Fig. 3: the variety “Mediterraneo,” a *sativa* Mediterranean type, after an early regrowth in late winter (not shown), grows less in spring than “Europe,” a Northern Flemish forage type, but in late summer and autumn, “Mediterraneo” grows more vigorously than “Europe.” In summer, “Tierra de Campos,” a wild *sativa* type, is the most vigorous of the four varieties. The “glomerata” type, a wild diploid accession, has a weak growth throughout the growing season.

A lucerne ideotype would combine a late spring regrowth that is related to a high autumn dormancy and a slow spring growth, both contributing to limit the competition with cereals. Those traits should be compatible with a root system pattern, that would limit its competitive impact for water. An erect growth habit would not be compulsory as in forage types. More studies are needed to test if creeping, rhizomatous and/or stoloniferous growth habit would be favorable against weeds without detrimental effect of cereals. In addition, as for lucerne forage varieties, varieties for living mulch should also be resistant to the main diseases and pests. Considering that seed production could be negatively affected by the introduction of wild genetic background in the breeding programs, much attention on seed yield production should be considered, while paying attention to select varieties without seed dormancy, a trait that is frequent in wild accessions (Ghaleb et al. 2021).

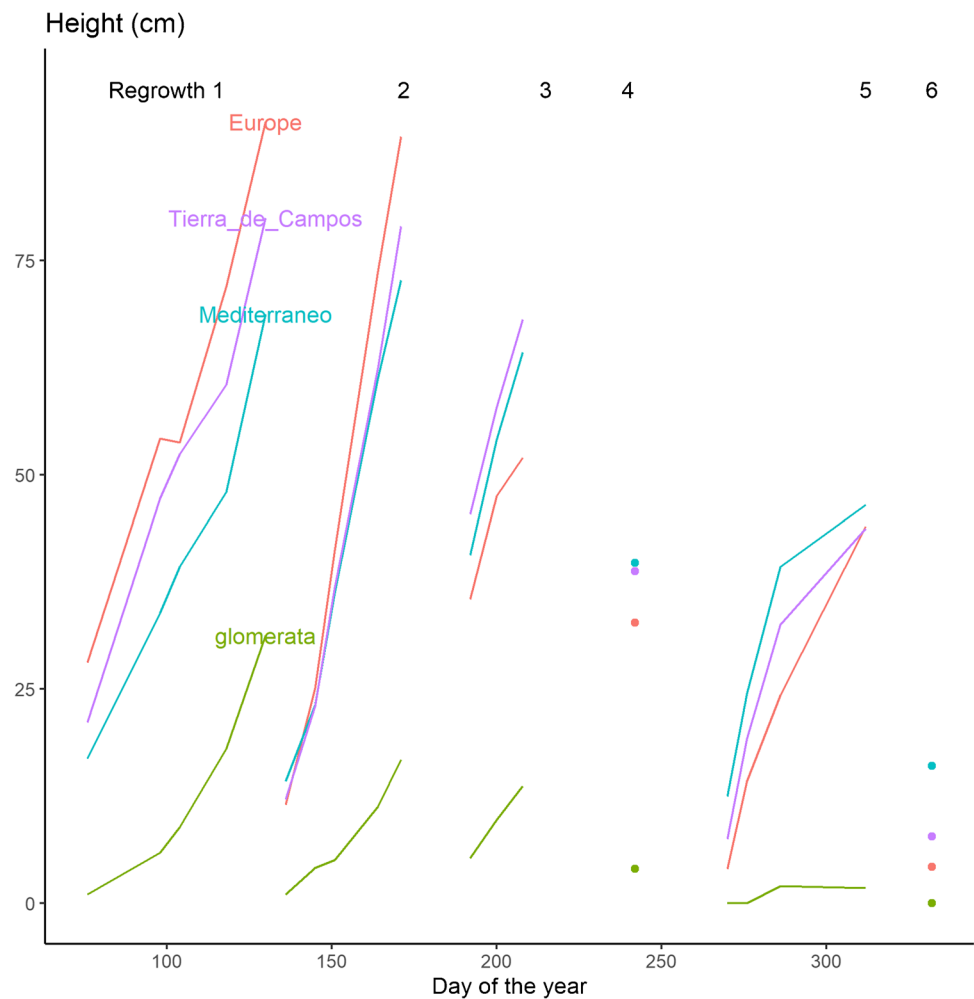
Genetic diversity for spring growth, autumn dormancy, disease and pest resistance, and seed yield is already described, but the genetic control of these traits is mostly known in erect, cultivated types of subsp. *sativa*. As in forage breeding, molecular tools would be helpful to increase genetic gains. For example, quantitative trait loci for key traits such as growth habit and autumn dormancy were identified in lucerne (Pecetti et al. 2021) in crosses between

Table 2 Yield of lucerne (*Medicago sativa*) populations belonging either to the subspecies *sativa* or *media*, either diploid (2×) or tetraploid (4×) or either wild (W) or cultivated (C) cut on five occasions

Subsp	Ploidy	Type	No	Spring yield (Mg ha ⁻¹)			Summer yield (Mg ha ⁻¹)			Autumn yield (Mg ha ⁻¹)			Total Yield (Mg ha ⁻¹)			Plant height (m)		
				Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
<i>sativa</i>	4×	C	14	4.2	1.1	7.6	8.6	3.7	12.7	1.5	0.8	1.9	14.2	5.6	21	0.75	0.53	0.94
	4×	W	3	2.9	2.7	3.1	3.5	2.3	4.7	0	0	0	6.4	5	7.7	0.55	0.50	0.59
	2×	W	1	3.5	3.5	3.5	2.3	2.3	2.3	0	0	0	5.8	5.8	5.8	0.58	0.58	0.58
<i>falcata</i>	4×	W	3	5.1	3.9	6.1	5.9	5.5	6.2	0	0	0	11	9.5	12.3	0.65	0.63	0.68
	2×	C	1	2.6	2.6	2.6	2.5	2.5	2.5	0	0	0	5.1	5.1	5.1	0.44	0.44	0.44
	2×	W	3	2.8	0.3	4.2	1.7	0	2.8	0	0	0	4.6	0.3	6.8	0.48	0.31	0.59

in 1994 (spring: 10 May; summer: 23 June, 04 August, 16 September; autumn: 08 November). Plant height was measured before the first cut on 10 May. Data from Julier et al. 1995).

Fig. 3 Evolution of canopy height of lucerne plants of different genotypes in six regrowth periods in the year 1994 in Lusignan, France. Dots represent regrowth periods in which height was measured just once (figure based on unpublished data of the experiment described in Julier et al. 1995).



subsp. *sativa* and subsp. *falcata* parents, offering prospects for marker-assisted selection. More genetic studies (genetic correlation among traits, heritability estimation, molecular markers associated to traits) are needed in the genetic backgrounds adapted to living mulch.

The opportunity to breed varieties dedicated to living mulch may be questioned. The farmers are often reluctant to buy seeds of a species that is not harvested and as such does generate direct economic income. Nevertheless, numerous varieties of cover crops such as white mustard (*Sinapsis alba* L.), fodder radish (*Raphanus sativus* L.), phacelia (*Phacelia tanacetifolia* Benth.), crimson clover (*Trifolium incarnatum* L.), and faba bean (*Vicia faba* L.) have been bred and are still bred by commercial companies, indicating that breeding efforts in “non-cash crops” are profitable. This demand for low-cost seeds is counter-balanced by the huge areas that are concerned: winter cereals are indeed the major arable crops under temperate climates. In such conditions, the cost of a breeding program aiming at releasing lucerne

varieties for living mulch could be economically cost-effective for a breeder.

The discovery of supernodulating plants in several legume species opens perspectives to breed varieties with an improved nitrogen fixation. These mutants lack the internal regulation of the production of the root nodules, which harbors the N fixing bacteria. In addition, N fixation in these mutants is less impacted by soil nitrate concentration. This trait results in a depressed biomass production due to the disproportion between the photosynthetic capacity of the shoot and the metabolic demands of symbiotic nodules (Novák 2010). Whereas this growth depression is problematic for forage production, it could be beneficial for legumes used as living mulch. The increased N fixation will lead for a higher N availability for the crop, whereas the decreased growth could reduce the competition with the crop.

Also in white clover, there is a large variation in morphology and phenology (Annicchiarico 2003) offering opportunities to breed varieties adapted for use as living mulch.

6.2 Ideomix breeding

While a first version of ideotypes for living mulch of perennial legume species are proposed, progress must be made by breeding both cereal and living mulch together. Eventually, breeding ideomixes, multi-species, and/or genotype mixtures selected for their agronomic performance in a particular environment could result in specific combinations of crop and mulch with a superior performance. In oats intercropped with lucerne (Nielsen et al. 1981) or berseem clover (*Trifolium alexandrinum* L.) (Holland and Brummer 1999), interspecific variety interactions for grain and forage production were found. Likewise, positive interactions between specific variety combinations of winter cereals and perennial legumes used as living mulch could be expected. Specific selection schemes were developed to create varieties of different species with an improved mixing ability (Sampoux et al. 2020). The combinations to be tested reaching numbers far larger than any breeding program might allow for pre-breeding of adapted genotypes and populations, as well as *in silico* testing of the combinations will have to be studied. This is why current individual-based models of legume mixtures have been designed by ecophysicologists, allowing to simulate the dynamics of the crop in response to light, water, and nitrogen (Faverjon et al. 2019). In such models, each individual is given a set of traits so that many virtual ideomixes can be tested *in silico*, in order to give the appropriate orientation of plant breeding schemes.

Finally, such methodologies would enable the breeders to test their candidate varieties *in silico* under many climatic years, including the changes expected with increasing atmospheric CO₂ concentrations, temperatures, and summer water deficits (Toreti et al. 2020). Indeed, it is expected that under moderate N fertilization, legumes should benefit more from the increase of atmospheric CO₂ concentration than C3 grasses so that varieties for 2050 might be significantly different from varieties adapted to current conditions.

6.3 Multi-species mulch

In forage production under low N, the use of species mixtures can lead to an increased biomass production. This is evident for grass–legume mixtures *versus* pure grass or legume forage stands under low N fertilization. Furthermore, a larger diversity in plant forms and phenology were shown to bring about more stable production with seasons and years (Prieto et al. 2015; Meilhac et al. 2019). Similarly, multi-species living mulch could offer advantages compared to a mono-specific living mulch. Especially in the period between two crops, when the living mulch acts like a cover crop, specific interactions could lead to greater biomass production or N fixation compared to a mono-specific cover like

it is the case for annual cover crops (Wendling et al. 2017; Rodriguez et al. 2021)

During the growing period of the cereal on the other hand, increasing inter- and intra-specific variation must not result in a living mulch with a greater ability to exploit the resources and in a stronger competition with the cereal crop. Indeed, the presence of various species and/or cultivar within the canopy might contribute to the required trade-off between fast covering the ground for preventing weeds and limiting maximum production for reducing competition for light. Further research is needed to elucidate whether increasing inter- and intra-specific variation in living mulch has agronomic advantages.

A last level of diversity could be used to tune even more precisely the living mulch–cereal interaction, using different varieties both for the cereal and for the living mulch. The resistance of multi-varietal cereal crops to various fungal diseases has been widely used in low pesticide input systems, for example wheat variety mixtures for resistance against *Septoria tritici* blotch (Kristoffersen et al. 2020).

Mixtures of cereal varieties grown on multi-species living mulch could hence play a role in the future of cropping cereals under climate under low herbicides, fungicides, and fertilizers inputs.

Depending on the ideotype defined for a living mulch, either a pure stand (one variety of one species) or a mixed stand (several varieties of one species or several varieties of several species) could be needed to combine all functional traits.

7 Conclusion

Despite the potential advantages of growing winter cereals on perennial living mulch such as soil conservation and N fixation, competition for resources results mostly in important cereal yield losses. Managing the competition between the crop and the living mulch is the cornerstone of the system. Managing this competition using current techniques remains tricky. Chemical or mechanical control techniques proved to reduce competition in several studies, but the environmental impact of these techniques might well undo the positive environmental impact of the living mulch. Other techniques such as adapted crop sowing densities or patterns also offer, *albeit* with a smaller impact than the former, opportunities to reduce living mulch competition. An option to reduce crop–living mulch competition that was not explored so far is to exploit the large genetic variation present both in the legume species that are used for living mulch as in the cereal crops.

We hypothesize that significant breeding effort can result in varieties of perennial legume crops that, when used as living mulch, will have a smaller impact on cereal yield

compared to cereals on living mulch of existing forage varieties. The ideotype of such varieties has a pronounced winter dormancy, starting its growth late in spring such that the cereal can take a lead in development in winter and spring. The living mulch should be short with a non-erect growth to limit competition for light with the crop. Seed production is abundant resulting in an acceptable seed price. Further breeding efforts to make the crop more competitive and using breeding schemes that improve the general mixture ability of both crop and living mulch could further reduce competition issues. Nevertheless, even with these dedicated varieties, competition between the crop and the living mulch might have a yield penalty compared to a wheat monocrop and management of this competition might remain necessary.

Many important questions remain unanswered after this review. Can growing cereals on living mulch be economically profitable? Can this system contribute to significant reductions of mineral N and pesticide use? Is there a positive effect on biodiversity when considering land sharing *versus* land sparing?

Growing cereals on living legume mulch is one among several options to reduce the dependency of arable crop rotations on N fertilizer and herbicides. Other options like ley arable crop rotations or use of mechanical weed control might be easier to implement at this stage to come to this end.

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