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Faculty of Natural Resources and Agricultural Sciences

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Inés Prieto Ruiz

Department of Crop Production Ecology
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Supervisor: Dr. Björn Ringselle. Swedish University of Agricultural Science. Department of Crop Production Ecology.

Assistant Supervisor: Professor Dr. Lars Andersson. Swedish University of Agricultural Sciences. Department of Crop Production Ecology.

Examiner: Professor Dr. Theo Verwijst. Swedish University of Agricultural Sciences. Department of Crop Production Ecology.

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Abstract

Weed control has always been a major challenge in arable lands. *Elymus repens* (couch grass), a rhizomatous perennial grass, is a particularly problematic weed in both annual and perennial crops in temperate climates. *E. repens* is dominant particularly in farming systems with no or limited use of herbicides. Ecological farming is in the rise due to the environmental and health awareness of consumers. Cover crops have been successfully used to reduce many weed species, but the effect on *E. repens* has been inconsistent and seemingly highly dependent on cover crop density.

The aim of this study was to investigate the effect that limiting resources and cover crops have on biomass allocation and acquisition of *E. repens*. To test these, two greenhouse experiments were conducted in 2014. In the first experiment, *E. repens* was grown under a combination of three light and three nutrient supply levels. Under decreasing light levels, *E. repens* shifted the allocation towards the above ground biomass and reduced the number of shoots, tillers and spikes; explained by the elongation of the stems seeking light. A decrease in nutrient availability increased the below ground (roots and rhizomes) fraction and decreased the number of tillers. Both treatments decreased total biomass compared to the control. In the second experiment, *E. repens* was grown under three seeding densities of red clover or perennial ryegrass. The use of both cover crops reduced the total biomass, number of aerial shoots and spikes of *E. repens*. Red clover did not change the allocation pattern while ryegrass increased the belowground mass fraction of *E. repens*, indicating that it competed strongly for nutrients.

In conclusion, biomass allocation and acquisition in *E. repens* was altered by abiotic and biotic factors.

Keywords: Couch grass, *Elymus repens* (L.) Gould, cover crops, competition, light, nutrients, weeds in organic farming, perennial weed.

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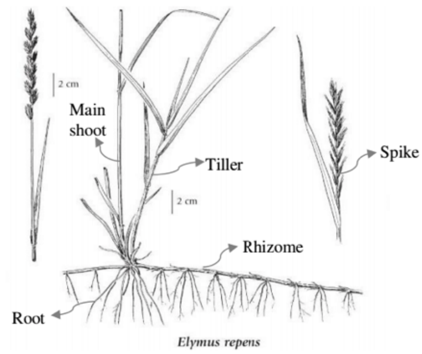
Popular Science

Organic farming is increasing due to the environmental and health awareness of consumers, and political decisions. However, weed control is a major constraint in this type of crop production since it does not allow the use of herbicides.



Couch grass *(Elymus repens)*, which is a weed highly present in the fields of Scandinavian countries, is a grass and, thus, from the same family as important cereal crops such as wheat, rye, oats and barley. It is a rhizomatous species, like ginger, with subterranean stems used for vegetative dispersal and storage of nutrients to survive winter. The belowground buds are located in nodes along the rhizome. Therefore, in order to control the plant it is key to control rhizome growth. A traditional technique generally practised in organic farming is repeated stubble cultivation, which fragments the rhizomes, stimulates regrowth and gradually deplete the rhizomes of nutrients. However, this intensive tillage is costly and increases the risk of soil erosion and nutrient leaching. Nutrient leaching is one of the leading causes of eutrophication and algae blooms (e.g. in the Baltic Sea).

The aim of this study was to find new ecological techniques to avoid the use of herbicides and tillage. One of these techniques is to sow cover crops, such as clover and ryegrass, together with the main crop. The cover crops compete for resources with weeds, primarily after the main crop has been harvested Moreover, in many cases cover crops improve the soil fertility, reduce soil erosion and increase biodiversity and ecosystem services, since they usually host different natural enemies of pest insects than the main crop.

In the first part of this study, we planted couch grass and artificially modified the light and nutrients, to understand how light and nutrient availability affect couch grass growth. We found that the reduction of light promoted a change in the allocation pattern from rhizomes to shoots, reducing the amount of rhizome present in the soil for the next generation. In the second part of the study, , we planted couch grass together with either red clover, a leguminous plant that fixates nitrogen from the air, or perennial ryegrass, another grass, which competes strongly for the nutrients in the soil. Both cover crops reduced the total biomass and the number of aerial shoots and spikes of couch grass. However, red clover did not change the allocation pattern, while ryegrass increased the rhizome fraction of couch grass.

Therefore, I conclude that, if utilized correctly, the under-sowing of cover crops in the crop rotation every year may significantly reduce the presence of couch grass in arable land.

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1 Introduction

Arable crops often suffer from a decrease in quality and yield due to weeds, insect attacks and plant diseases, causing high economic losses. *Elymus repens* (L.) Gould (1947), a highly competitive rhizomatous weed in temperate climates (Central Europe, Scandinavia, Russia and North America) (Fig. 1), is considered one of the most problematic weeds, reducing profits in both annual (e.g. corn, oats, soybean, wheat, barley or rye) and perennial (e.g. alfalfa) cultivated crops due to its effective competition for key nutrients (Westra and Wyse, 1981). As a result, a great deal of study has been performed on *E. repens* since the beginning of the 20^{th} on its biology and methods to control it.

Elymus repens (L.) Gould is a geophyte of the Poaceae family, also known as *Elytrigia repens* (L.) Nevski (1933), *Agropyron repens* (L.) Beauv. (1812), or *Triticum repens* L. (1753) but it is commonly called couch grass or quackgrass. Although *E. repens* is native from Europe, it can be found between the tropics and the Polar Regions or at higher altitudes with cool climates in warmer regions (Håkansson, S., 1982, 1969a; Holm *et al.*, 1977). It may usually be found on sunny open agronomic areas and on field, road or river margins (Palmer and Sagar, 1963).

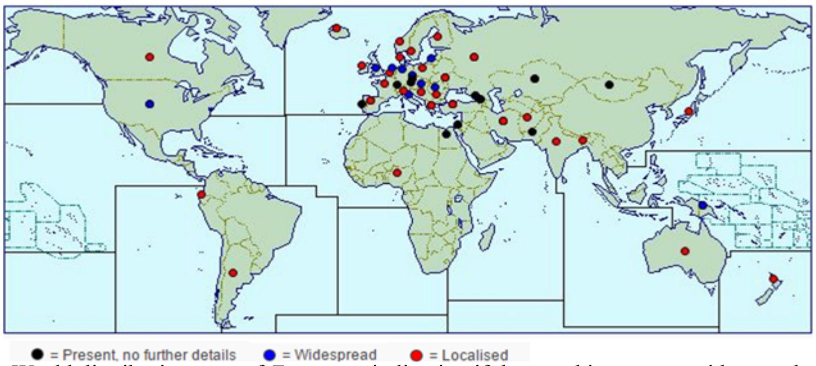

Figure 1. World distribution map of *E. repens*, indicating if the weed is present, widespread or has been localized. Figure by cabi.org.

Elymus repens is known as a “cool-season” plant, growing preferentially in areas with moderate rainfall (Werner and Rioux, 1977) and is being also drought and frost tolerant (Palmer and Sagar, 1963). As a C3 plant, it does not adapt well to hot dry climates (Håkansson, S., 2003) and, all growth is depressed at temperatures higher than 35 ºC (Bond, W., 2006). It tolerates soils with pH between 4.5 and 8.0, preferring light neutral to alkaline soils (pH 6,5-8.0) (Dale *et al*., 1965). It also has a high salt tolerance (70-95 mg/100 g of soil) (Werner and Rioux, 1977).

Elymus repens is a perennial grass that reproduces sexually by seeds or vegetative by rhizome propagation, which is more important in open communities (Bond, W., 2006). Rhizomes are storing subterranean stems, where assimilates are stored and buds are located. They are smooth and pale yellow with a tough brownish sheath at nodes. Rhizomes grow up to one meter forming an extensive system, usually between the first 20 cm of the soil, never deeper than 40 cm. Growth is renewed annually during late spring to late summer from lateral buds that develop on the base of aerial shoots or on the nodes of existing rhizomes (Palmer, 1958; Håkansson, 1982). All mature buds can produce a new plant, but already established shoots suppress the buds through apical dominance, unless the shoot is destroyed or the rhizome network is fragmented (Werner and Rioux, 1977).

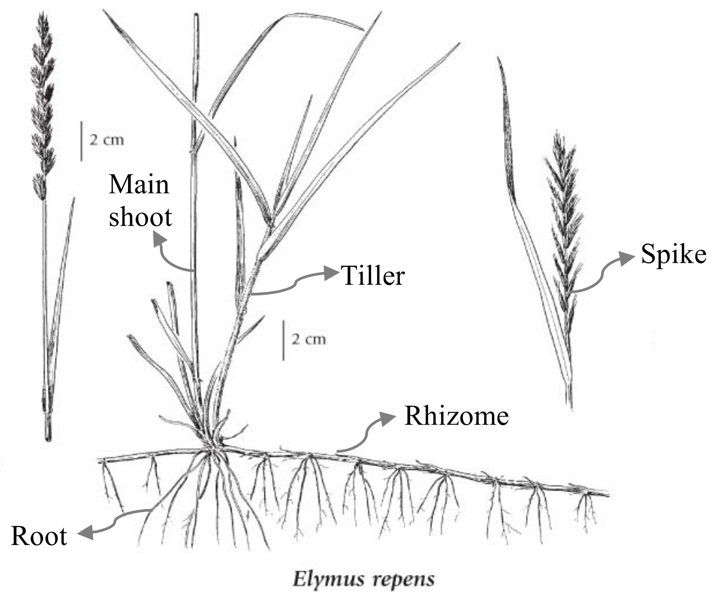

Figure 1. Elymus repens diagram. Parts of the plant with theirs names. Figure by Illustrated flora of British Columbia (Douglas *et al.*, 2001)

Rhizome tips grow horizontally during spring and summer, becoming erect in autumn (Evans M. W., 1935). These primordial aerial shoots grow slowly over winter until soil temperature is over 0 ºC (late March) when growth is reactivated. Side aerial shoots called tillers are produced from rhizomes when the main shoot is in the 6-8 leaf stage (Palmer and Sagar, 1963) (Fig. 2). In June-July spikes are formed at the top of the stems, producing a variable amount of seeds due to self-sterility and wind pollination among other reasons (Werner and Rioux, 1977). Seeds ripen in late August, and drop from the plant in late September. The point at which the production of energy is the same as respiration (compensation point) and consequently when the rhizomes shifts from energy source to energy sink occurs at the 3-4 leaf stage in unshaded plants (Håkansson, S., 1969a)

Even though *E. repens* is considered a weed it can have many beneficial effects. It may be used for forage or hay since it has a good balance of nutrients for cattle, and therefore, not causing great damage in grasslands. The underground system protects well against erosion on slopes or bare soils, and provide cover for wildlife (Werner and Rioux, 1977).

Elymus repens was traditionally controlled with mechanical methods and during the green revolution, especially in the '60 and '70, many of studies were conducted to optimize chemical and mechanical control (Cussans, 1972; Palmer and Sagar, 1963; Rioux *et al*., 1974; Waterson *et al*., 1964).

From the seventies, pronamide (no longer allowed in the EU) but mainly glyphosate (N-(phosphonomethyl) glycine), a non-selective broad-spectrum systemic herbicide, has been widely used due to its high effectiveness in conventional agriculture. Mechanical options, especially tillage, are very useful for perennial weeds control. Tillage, specially stubble cultivation and ploughing, is meant to kill the shoots and fragment rhizomes burying them deeply enough to avoid sprouting, or pulling them to the surface to dry out. Nonetheless, rhizome fractions might not be buried deep enough or pulled out completely, causing the activation of buds and the spout of new shoots, increasing the presence of the weed (Håkansson, S., 1967; Melander *et al*., 2013). Both herbicide use and tillage can cause environmental issues. Chemicals can contaminate soil and water, whereas mechanization, despite its advantages, increases costs associated with machinery and energy as well as environmental damages such as nutrient leaching, emission of pollutant gases and/or soil compaction.

Recently, costumer concern over health risk and environmental degradation has increased, leading to a rise in the number of organic farms. The European Union has published the directives 2007/834/EC and 2008/889/EC, regulating organic farming. According to this directive, the use of synthetic pesticides or herbicides are only allowed in preventing the development of weeds as a last resource. The change in consumption patterns and the consequent laws promoted new studies on crop rotation, living mulches, cover crops, optimal mechanical control or a combination of the latter two in the suppression of weeds. (Bergkvist *et al*., 2010; Brandsæter *et al*., 2012; Melander *et al*., 2013; Rasmussen *et al*., 2014; Ringselle *et al*., 2015).

From these new ecofriendly and cost-effective strategies to fight weeds and improve soil quality, cover crops are gaining great importance in the market. Cover crops are crops planted in agroecosystems for different ecological benefits other than profitable crops (Upadhyaya and Blackshaw, 2007). They may be sown into the main crop or after its harvest. Their main advantages are related to quality of the soil, reduction of erosion, increment of soil fertility, especially if they are leguminous, and improvement of soil structure and moisture. Other main features of cover crops are reduction of weeds, both annual and perennial, of bacterium and fungi populations (Hartwig and Ammon, 2002) or act as trap crops to reduce pest on the cash crop. On the other hand, they also cause losses of the main crop due to competition for resources. If left after harvest, cover crop residues may interfere with the correct establishment of cash crop seeds or release allelopathic substances (Upadhyaya and Blackshaw, 2007). The effect may differ between experiments due to the establishment degree of the cover crop or allelopathic effects (Brandsæter *et al.*, 2012; Melander *et al*., 2013; Palmer and Sagar, 1963).

In the temperate zones the most effective and common practice to reduce *E. repens*, when using living cover crops is to under-sown them in the main crop. Such as clover or a combination of clover and grass or of grass and catch crop (Ling Zou, 2014; Upadhyaya and Blackshaw, 2007; Anderson, 2015), grown to reach maximum competitiveness of cover crops at *E. repens* compensation point (Håkansson, S., 2003).

To potentially control *E. repens*, cover crops have to be able to compete with it by reducing its total biomass, but especially its rhizome biomass. Consequently, cover crops which are able to change the allocation pattern of *E. repens* away from the rhizomes may be especially suppressive. Reducing important competitive features such as tillers and shoot number may also indicate that a cover crops is especially competitive against *E. repens*

Environmental conditions influence whether buds will to turn into shoots or rhizomes (Cussans, 1972). E. repens is a shade sensitive plant, when growing under limiting light E. repens reduces rhizome dry weight towards the above ground biomass (Poorter and Nagel, 2000; Skuterud, 1984). Williams (1970) found that shading reduces rhizome dry weight by half as well as increases aerial stem length and the amount of leaves. Thus, rhizome growth has been shown to be much more sensitive to the level of illumination than aerial growth (Håkansson, S., 1969a; Palmer J.H., 1958). E. repens has high plasticity to light which makes it more tolerant for competition (Callaway et al., 2003) and can change its competitive strategy from a guerrilla strategy to a phalanx strategy, by morphological variability of aerial organs (Amiaud et al., 2008).

The availability of nutrients and water also changes the allocation pattern, limiting them causes the shift of photoassimilates towards roots (Poorter and Nagel, 2000). Under decreasing nitrogen supply on the soil, above ground production is reduced (McIntyre, 1965) while the concentration of potassium or phosphorus does not have an impact on shoots or rhizomes (Bandeen and Buchholtz, 1967). Allelopathy also shifts the resources towards the belowground (Ninkovic, 2003; Rutherford and Powrie, 1993).

Red clover *Trifolium pratense*. L. (cvar. 'Ares') and ryegrass *Lollium perenne* L. (cvar. 'Birger') are two with similar life cycle that commonly used cover crops in cereal fields in Scandinavia which will potentially reduce the presence of *E. repens* in the crop fields.

Red clover is an herbaceous perennial deep taproot plant with a dense canopy composed by horizontal trifoliate leaves which shade *E. repens* leaves and may therefore effectively compete for light. Although red clover competes for water and poorly nutrients, red clover does not compete for nitrogen since, as a leguminous, can fix air nitrogen. As a consequence, red clover is often used not for its competitive ability, but for its tendency to increase the nitrogen level for the subsequent cash crop (Bergkvist *et al.*, 2011). Ryegrass, a cool season perennial Poaceae with an extensive root system produces a great amount of biomass and empties the top soil of nutrients (Breland, 1996).

This study will help to understand new strategies to control *E. repens* in organic farms where no chemical inputs are allowed.

2 Aim and Hypothesis

2.1 Aim

The aim of this study was to investigate how light and nutrients limitations, as well as cover crop competition, affect *E. repens* biomass acquisition and allocation, to potentially improve the efficacy of cover crops as a control method for *E. repens* and other perennial weeds.

2.2 Hypothesis

- Limiting light and nutrients will reduce the total biomass of *E. repens*.
- Allocation of resources will change when growing under different light intensities, increasing the above ground biomass on lower light intensities.
- Nutrient availability will affect allocation of resources, shifting from above ground biomass to below ground organs on decreasing availability.
- Cover crops will reduce *E. repens* total biomass and change the allocation pattern.

3 Materials and Methods

Two greenhouse experiments were conducted simultaneously at Ultuna, 5 km south of Uppsala, Sweden (59°49'05.0"N 17°39'23.7"E) from the 11th of February to the 4th of May 2014, to analyze the different allocation of resources in *E. repens* under different light and nutrient conditions or in competition with perennial ryegrass *L. perenne* and red clover *T. pratense*.

3.1 Experimental design.

In the first experiment, light and nutrients were fixed at three different levels. Light treatment was divided into three shading intensities, maximum light (100% light intensity - uncovered pots, HL), medium light (75% light intensity - pots in a rigid plastic cage, ML) and low light (50% light intensity - pots in cage with lid, LL) (Fig. 3), reduction of light on the pots due to the cages was measured with a light meter (SKR 1800 2-Channel Light sensor, Skye). Nutrient availability was also divided into three doses, high nutrients (HN), medium nutrients (MN, 50% of HN) and low nutrients (LN, 25% of HN). Light and nutrient treatments were applied simultaneously and as a combination of factors (For fertilized water solution see section 3.4).

In the second experiment, competition was provided by two different cover crops, ryegrass (*L. perenne*) and red clover (*T. pratense*). Cover crops were seeded independently in three different densities, 6, 12 and 24 plants per pot (136.36 seeds m^{-2}, 272.72 seeds m^{-2} and 545.45 seeds m^{-2} respectively). The cover crop pots were under the same conditions as High light - High nutrients from experiment one to enable comparisons with the monoculture.

Both experiments were conducted at the same time, under the same external conditions and integrated into seven blocks which were established in two correlative greenhouses. Each block contained two randomized arranged replicates of each treatment, one for each harvest. The randomized factor prevented the pots from bias between them. Each replicate represented one of the two harvested that were performed. A total number of 210 pots were placed on six tables (4.5 m by 1.6 m) with a separation between pots of 30 cm. Sowing was done on the 11th of February and the application of light and nutrient limitation started on the 28th of February 2014.

Table 1: *Treatments: Light and Nutrients interaction. Cover crops densities. Number of pots of each treatment at the beginning of the experiments.*

First Experiment			**Second Experiment**		
Light Level	**Nutrients Level**		**Cover crop**	**Cover crop seed density**	
Low: Cage with lid	Low: 25 %	LL-LN	Red clover	6	C6
Low: Cage with lid	Medium: 50 %	LL-MN	Red clover	12	C12
Low: Cage with lid	High: 100 %	LL-HN	Red clover	24	C24
Medium: Cage	Low: 25 %	ML-LN	Ryegrass	6	R6
Medium: Cage	Medium: 50 %	ML-MN	Ryegrass	12	R12
Medium: Cage	High: 100 %	ML-HN	Ryegrass	24	R24
High: Uncovered	Low: 25 %	HL-LN			
High: Uncovered	Medium: 50 %	HL-MN			
High: Uncovered	High: 100 %	HL-HN	Control (HL-HN)		

3.2 Rhizome preparation of *E. repens*.

The rhizomes used for both experiments were collected on November 12th 2013 from organically cultivated arable land close to Knivsta, Sweden (59°44'13.0"N 17°38'58.9"E). In order to have the most homogenous population of *E. repens* possible, they were propagated in a greenhouse for one month and a half.

On the 7th of February 2014 rhizomes were harvested and washed up with warm water to remove soil and dirt. Unhealthy rhizomes were rejected, and the rest were cut into 8 cm pieces, each with between 2 and 4 nodes. Each fragment was weighed and divided into 3 classes 0.25-0.3 g, 0.3-0.4 g and 0.4-0.45 g.

3.3 Planting.

Prior to planting, 210 plastic boxes (26.5 cm x 16.5 cm x 14 cm, 0.0044 m^2) were washed. Each pot was filled with approximately 4 liters of pumice stone, 70% of the total volume of the pot. The soil used was “Hekla Green Pumic stone” (Bara Mineraler), (SiO_2 (71% of dry weight), Al_2O_3 (15.5%), Fe_2O_3 (5%), Na_2O (4%), K_2O (3%), CaO (2.8%)). Particle size was on average 1.5 mm. This soil was selected because it is lightweight, has a low buffering effect, excellent drainage, it was not fertilized and did not contain organic material, as well as for its convenience to extract the roots.

On the 11th of February 2014, one rhizome piece of each category was evenly planted at a 2 cm depth on every pot, so the total rhizome weight was 1 gram fresh weight/pot for both experiments. Every pot received 1500 ml of fertilized water. In the second experiment, once rhizomes were planted, ryegrass and red clover seeds were sown on the wet surface and covered with a dry thin layer of soil. To guarantee germination, the seeds were sown with a 25% increase of the required doses and, 10 days later, seedlings were counted and any superfluous plants were pulled up manually to maintain the pre-established doses in each treatment. To enable the fixation of air nitrogen, red clover seeds were inoculated with the Rhizobium bacteria (Inocula Scandinavia).

Cover crops emerged on the 21st of February. The first visible sights of emergence of *E. repens* was on the 24th of February.

Figure 3. Details of the pots. From both experiments. Different light treatments: Uncovered pots, pots in cage and in a cage with a lid. As well as pots with cover crops. Photo: Inés Prieto SLU

Figure 4. Details of the plant: shoots, rhizomes and roots. Photo: Björn Ringselle SLU

3.4 Watering and fertilization treatments.

Fertilized water solution was produced by diluting Wallco nutrient solution 51-10-43 + micro (Cederroth International AB, Sweden) to 0.196 % in high nutrients. (100 mg N l^{-1}, 19,6 mg P l^{-1}, 84,3 mg K l^{-1}, 7,84 mg l^{-1}, 7,84 mg S l^{-1}, 5,88 Ca l^{-1}, plus other microelements with a concentration below 3,92 mg l^{-1}.). Fertilized irrigation water was then further diluted with deionized water in the medium and low nutrient treatments to 50 and 25% of the high nutrient treatment, respectively.

Pots were regularly watered every 3-4 days with 600 ml of water with nutrients levels according to treatment and rotated anti-clockwise within the block to minimize bias from placement. Towards the end of the experiment additional watering with nutrient-free water was performed to avoid drought effects due to the ever increasing biomass. For the first two weeks all pots were given the same amount of nutrient water (for a total of 0.35 grams of nitrogen/pot) and, afterwards according to treatment. Therefore the total nitrogen applied was for the first harvest 0.83 gr on plants with cover crop or high nutrient treatment, 0.59 gr on medium nutrient treatment and 0.47 gr on low nutrient treatment, and 1.25 gr, 0.8 gr and 0.575 gr respectively by the end of the second harvest.

3.5 Greenhouse conditions.

Temperature in the greenhouses was set to 18 °C during the day (08.00-24.00) and 8 °C during the night (00:00-8:00) and humidity was set to about 70 %. However heat from the lamps and the cool down period between night and day generally meant that, on average, the temperature was a few degrees higher than the set values and the humidity about 50%, during the day (Fig. 5).

Artificial light was provided by 400 W lamps (Two Master HPI-T and five Master SON-T PIA Plus per row, Koninklijke Philips N V, Netherlands) which were on during the day, resulting in about 200 μmol s^{-1} m^{-2}. These lamps were placed 50 cm above the pots and automatically turned off if the insolation exceeded 1000 μmol s^{-1} m^{-2}, which happened some days towards the end of the experiment.

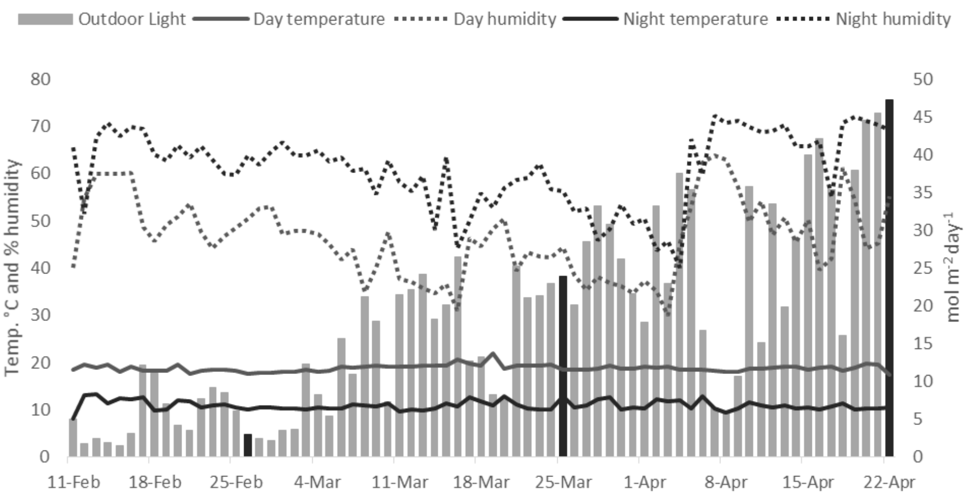

Figure 5. Temperature and humidity during day (08:00-24:00) and night (00:00-08:00) Bars show the total daily insolation measured just outside the green house. Dark bars indicate the start of the light and nutrient treatments, the first and the second harvest (Figure from Ringselle, 2015).

3.6 Harvesting and sampling procedure.

The first harvest started on the 25th of March (day 42). Both experiments were harvested at the same time following the same procedure. Harvest was performed block-wise, with the pots from each block being collected from the greenhouse and harvested within the same day. *Elymus repens* above ground biomass was separated from the below ground biomass. The number of main shoots (the ones that emerged from an independent node) and tillers of each rhizome were counted and recorded. The belowground biomass was divided into new rhizomes, old rhizomes and roots. The cover crops were separated from the *E. repens* and divided into aerial and subterranean biomass.

Elymus repens shoots were dried in an oven for 24 hours at 60 ºC to keep the nitrogen intact while all the other biomass, was dried for 24 hours at 105 ºC. Dry samples were weighed and recorded.

The second harvest took place between the 22nd of April (day 72) and the 4th of May following the same method as the first. In this harvest spikes present in the *E. repens* were also recorded. Nitrogen and carbon concentrations of the *E. repens* shoots from the second harvest were analysed by combustion on an elemental analyzer (Leco CNS-2000, Leco Corp., MI, USA; Kirsten & Hesselius 1983).

3.7 Statistical analysis.

The statistical analyses described in the following section were evaluated in linear mixed model with main effects being Light, Nutrients and the Interaction of light and nutrients, for the first experiment, and Red clover and Ryegrass for the second experiment, plus their interactions as fixed effects and Block as a random effect. Although each experiment was analyzed independently, High Light-High Nutrient (HL-HN) treatment from experiment one was used as control for both experiments. Analyses were performed for each harvest independently.

The allocation pattern was analyzed also as a fraction due to the advantages explained in Poorter and Sack, 2012. Significant results for the different variables are those with a P value below 0,05. Tukey tests were also performed to study the significance between treatments.

All analysis was done with the computer program JMP Pro 11 (SAS Institute Inc.)

Data was divided into groups of parameters:

- Dry weight, the weight of the biomass after it was dried.
- Fractions, dry weight of the parameter as a portion of the total dry weight of the pot was studied to understand which part of the plant *E. repens* prioritized when under different light and nutrients conditions.
- Red Clover cover crop.
- Rye Grass cover crop.

Variables (main effects):

- Light, plants were subjected to three different artificial light intensities (LL-Little (50%); LM-Media (75%) and LH-Full (100%). Light was used to comprehend the response of *E. repens* in privation of light and then, this data used for the cover crops competition study.
- Nutrients, to understand the implications of the nutrients on *E. repens* growth pots were subjected to three artificial nutrients levels. (NL-Low (25%); NM-Medium (50%); NH-High (100%))
- Interaction, the combination between light and nutrients were used because they offered a closer indication of the interaction of both variables.
- Repetitions were set in blocks, for the statistical analysis they were set as a random effect.

4 Results.

4.1 Light and nutrients artificial modification.

In the first experiment, light intensity and nutrient availability were modified artificially to study the effects on total biomass and how that biomass was allocated in *E. repens* plants.

Table 2 *Results of the analysis of variance (ANOVA) for the first experiment showing the effects of Light (L), Nutrient (N), their Interaction (I) and their interaction on E. repens, dry weights (dw) and fractions in Harvest 1 and 2. Bold letters indicate P-values smaller than 0.05 (Significant values).*

		Root dw	New rhi-zome dw	Shoot dw	Old rhi-zome dw	Total dw	Roots fraction	New Rhi-zome fraction	Shoots fraction	Old Rhi-zome fraction
	DF	P	P	P	P	P	P	P	P	P
Harvest 1										
Light	2	**<.0001**	**<.0001**	**<.0001**	**0.0107**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Nutrients	2	0.9759	0.6173	0.2805	**0.0033**	0.5262	**0.0102**	0.5789	**0.0213**	0.1761
Interaction	8	**<.0001**	**<.0001**	**0.001**	**0.0074**	**<.0001**	**<.0001**	**<.0001**	**0.001**	**0.001**
Harvest 2										
Light	2	**<.0001**	**<.0001**	**<.0001**	0.1195	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Nutrients	2	0.7415	0.0712	**<.0001**	0.579	**<.0001**	**<.0001**	**0.0001**	**<.0001**	**0.0042**
Interaction	8	**<.0001**	**<.0001**	**0.001**	0.4093	**<.0001**	**<.0001**	**<.0001**	**0.001**	**0.001**

Table 3 *Results of the analysis of variance (ANOVA) for the first experiment showing the effects of Light (L), Nutrient (N), their Interaction (I) and their interaction on E. repens: shoot, tiller and spike counting for both harvest. As well as Carbon and Nitrogen percentage on the shoots of the second harvest. Bold letters indicate P-values smaller than 0.05 (Significant values).*

		Total	Main shoot	Tiller	Spikes	Tot-C % av ts	Tot-N % av ts
	DF	P	P	P	P	P	P
Harvest 1							
Light	2	**<.0001**	**0.0261**	**<.0001**			
Nutrients	2	0.5489	0.6547	0.5072			
Interaction	8	**<.0001**	0.0524	**<.0001**			
Harvest 2							
Light	2	**<.0001**	**0.002**	**<.0001**		**<.0001**	**<.0001**
Nutrients	2	**<.0001**	**<.0001**	**<.0001**		**0.0395**	**<.0001**
Interaction	8	**<.0001**	**<.0001**	**<.0001**	0.2327	**<.0001**	**<.0001**

Reduction of *E. repens* total biomass under limiting light and nutrients.

Limiting light and nutrients caused a reduction in *E. repens* total dry weight compared to the control (Fig. 6). By the time of the first harvest, *E. repens* had a shorter period of time to grow under the limiting factors, showing significant differences in total biomass growth when light was limited. This reduction on total biomass, however, was steeper in the second harvest. When growing under low or medium light level, light was the limiting growth factor, even though the increasing availability of nutrients set a non-significant rising trend. For plants exposed to full light (HL), the limiting factor was the nutrient availability.

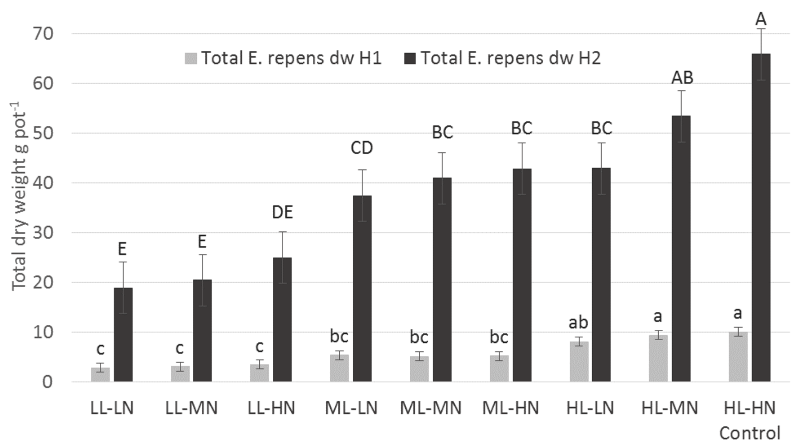

Figure 6. *E. repens* total biomass as the interaction of light and nutrients in grams per pot in harvest 1 and 2. Error bars are 95% confidence interval and letters Tukey HSD test comparisons.

Effects of limiting light intensity and nutrients availability in the allocation of resources.

Modifying light had a significant impact on the growth of the different structures of *E. repens* as presented in the ANOVA tables (Tables 2 and 3). Decreasing light intensity reduced mass in all organ types, while lower nutrient availability primarily reduced shoot biomass compared to the control in both harvests (Fig. 7 A and B). Dry weight fractions showed an increase of the aerial shoot fraction when growing under lower light intensities, while allocation shifted toward the roots and new rhizome on decreasing nutrient availability (Fig. 7 C and D).

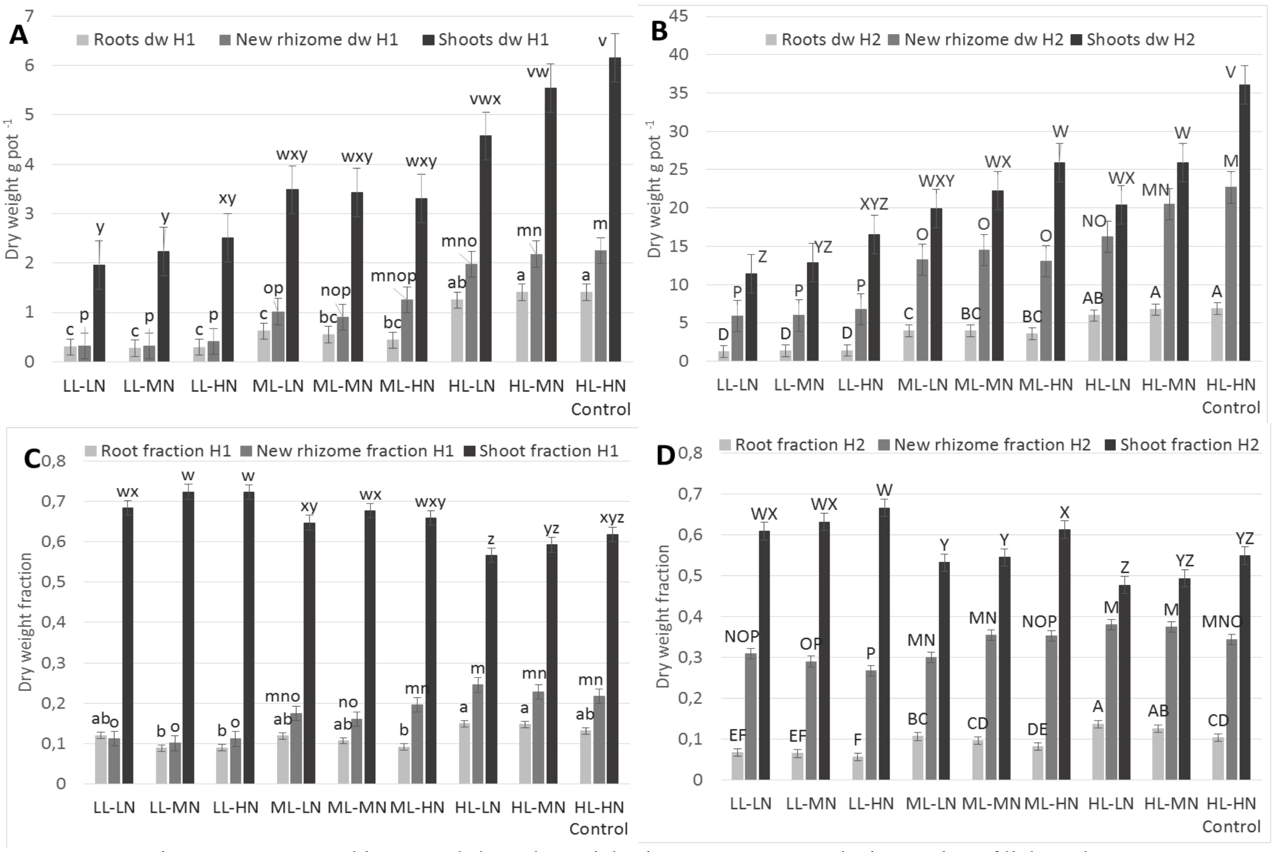

Figure 7. Root, new rhizome and shoot dry weights in grams per pot as the interaction of light and nutrients on harvest one (A) and two (B). Root, new rhizome and shoot fraction in harvest one (C) and two (D). The letters for the Tukey Test analysis follow different parts of the abecedary for the different organs (Please note the difference in scale in the Y-axes between harvests)

The amount of aerial biomass produced was related to light intensity and nutrient availability. This trend was greater in the second harvest as plants had a longer period of growth and were exposed to the treatments for a longer time. *E. repens* shifted resources towards its above ground biomass when more nitrogen and more light for the three light levels were applied, with the control (HL-HN) significantly different from the other levels (Fig 7 B). When comparing the HL-MN with the control, nutrients were the limiting factor in the same light level. Overall light was a greater limiting factor than nutrients (Fig 7 B).

The root fraction was higher in the first harvest than in the second, and even though roots grew more under higher light intensities, they also developed more when fewer nutrients were available. The rhizome (old and new) fraction was higher as light intensity increased and nutrients decreased, for both harvests (Fig. 7 C and D).

Shoot fraction followed the same pattern in both harvests. It increased when plants were provided with more nutrients and decreased with increasing light intensity (Fig. 7 C and D).

Limiting light.

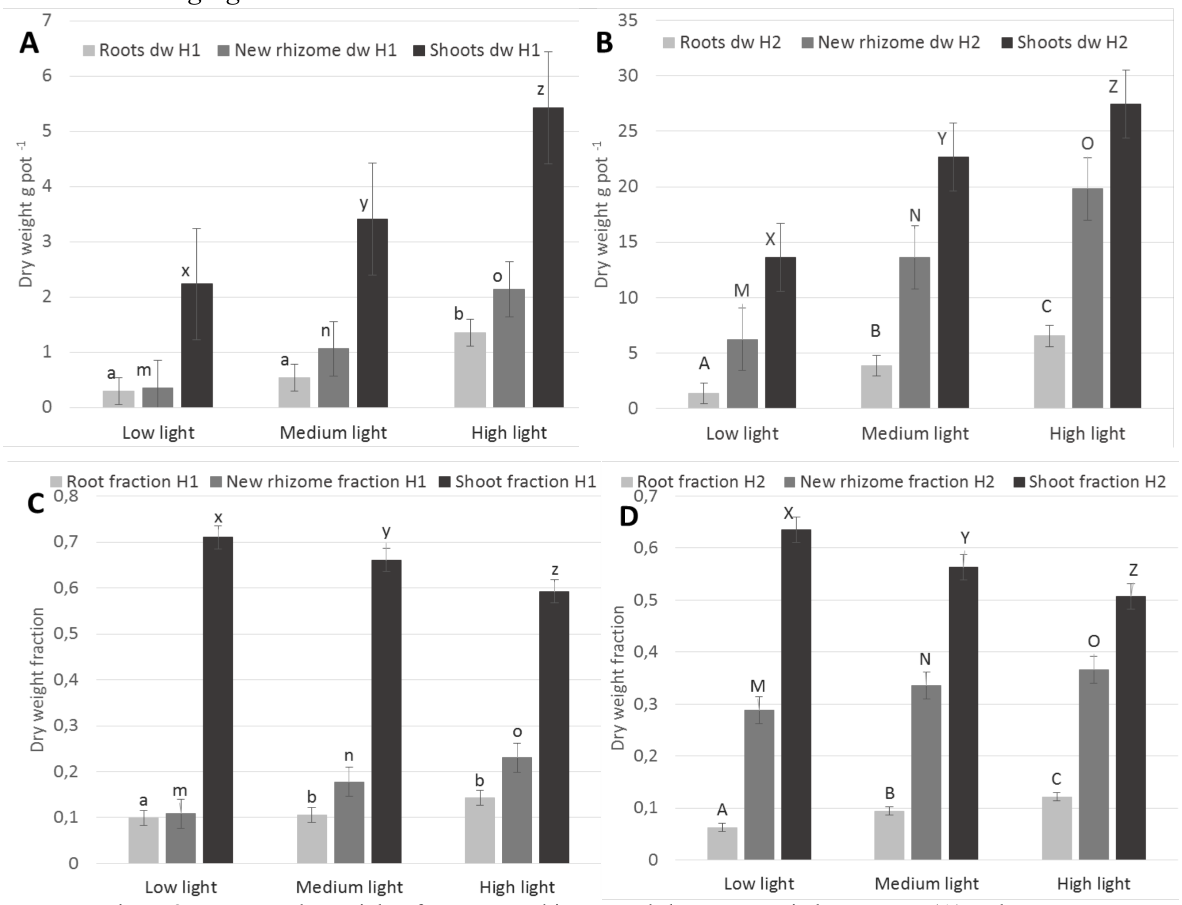

Figure 8. *E. repens* dry weight of roots, new rhizome and shoot per pot in harvest one (A) and two (B) due to light limitations. And allocation of *E. repens* on roots, new rhizome and shoots as fraction of the total dry weight when growing under limiting light. Percentages per pot in harvest one (C) and two (D). (Please note the difference in scale in the Y-axes between harvests)

Root, new rhizome and shoot growth decreased with decreasing light intensity. New rhizome and shoot dry weights were significantly different in the three light intensities for both harvest (Fig. 8 A and B). Old rhizome dry weight reduced with decreasing light (data not shown). Dry weights were significantly higher in the second harvest than in the first one for roots, new rhizomes and shoots dry weights.

The biomass fraction varied with light intensity and harvest. As light decreased, allocation of resources moved towards the shoot fraction rather than to below ground (roots and rhizome) (Fig. 8 A and B). In the first harvest, *E. repens* was half of that in the second harvest (Fig. 8 A and B). Dry weights and fractions were significant for light in both harvests (Table 2).

Limiting nutrients.

Low and medium nutrient availability resulted in a lower shoot dry weight compared to high nutrient availability, but only in the second harvest (Fig. 9 A and B). Roots and new rhizome biomass was not affected by nutrient availability, but old rhizome dry weight decreased with increasing nutrient availability.

Resource allocation shifted towards rhizomes with lower nutrient availability. Root fraction also increased as nutrient supply decreased although the fraction did not change between harvests. The old rhizome fraction declined as nutrient availability increased (data not shown). Shoot fraction was higher when more nutrients were applied to the plants, displaying a significant difference in high nutrients availability (Fig. 9 C and D).

The interaction between light and nutrient was significant for root and new rhizome dry weight. More light led to more rhizome formation although it was limited by the amount of nutrients. Root growth increased as the plants were exposed to more light regardless of the amount of nutrients given at each light level (Fig. 7 A and B).

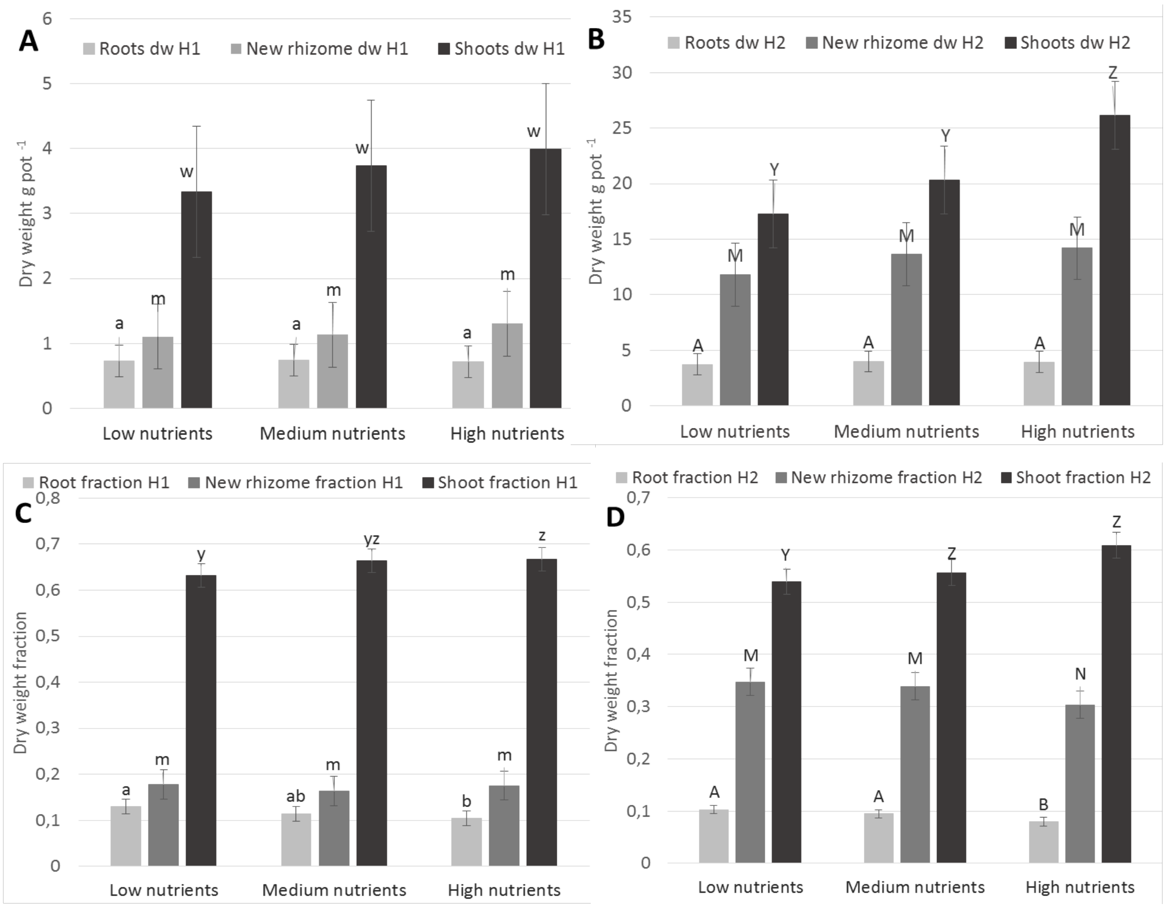

Figure 9. Dry weight of roots, new rhizome and shoot per pot in harvest one (A) and two (B) due to nutrient limitations. Allocation of *E. repens* on roots, new rhizome and shoots as fraction of the total dry weight when growing under limiting nutrients. Percentages per pot in harvest one (C) and two (D). Please note the difference in scale in the Y-axes between harvests.

Old rhizome dry weight followed an increasing trend correlating to increasing light levels as well as a decreasing trend in decreasing nutrient levels. These trends were steeper in the first harvest and in the nutrients levels. Old rhizome dry weight was lower in the first harvest than in the second (Figure not shown).

Concentration of carbon in the shoots increased with increasing light intensity. Under high light (HL), it increased as nutrient application was augmented, whereas for the other light intensities, nutrient application did not show an effected (Fig. 10 A). Nitrogen concentration diminished with increasing light intensity and grew with increasing nutrient availably (Fig. 10 B).

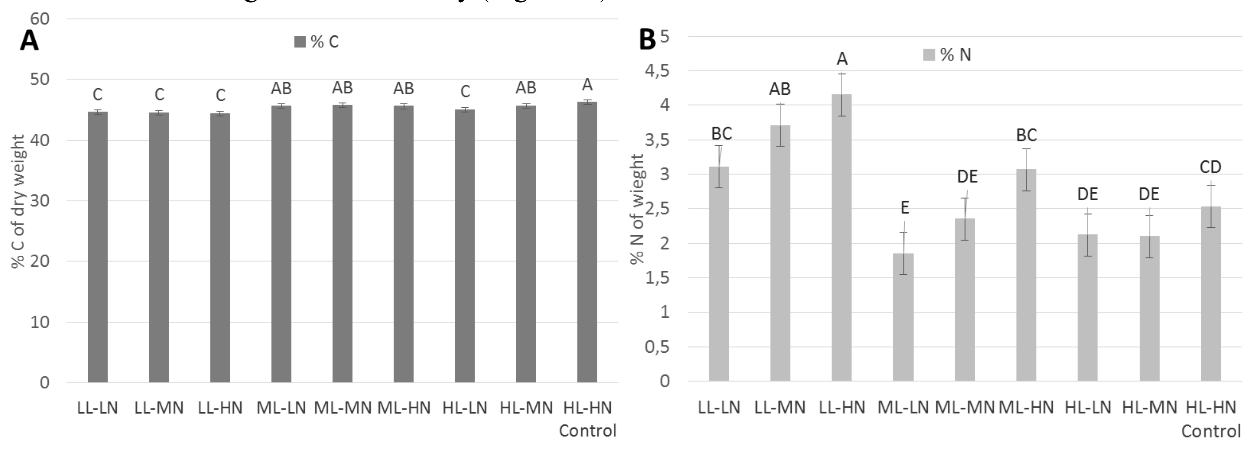

Figure 10. Carbon (C) (A) and Nitrogen (N) (B) concentrations in *E. repens* shoots (% of dry weight) when growing under different light and nutrients interactions. (Measurements in the second harvest).

Morphological features under limiting resources.

E. repens produced a higher number of shoots and tillers as light intensity and nutrient availability increased (Fig. 11 A and B), however in the second harvest, plants under high light or nutrients level displayed higher shoot production than the other treatments. Spikes only developed in the second harvest under high light no matter the nutrient condition and under medium light only at high nutrients level (Fig. 11 C).

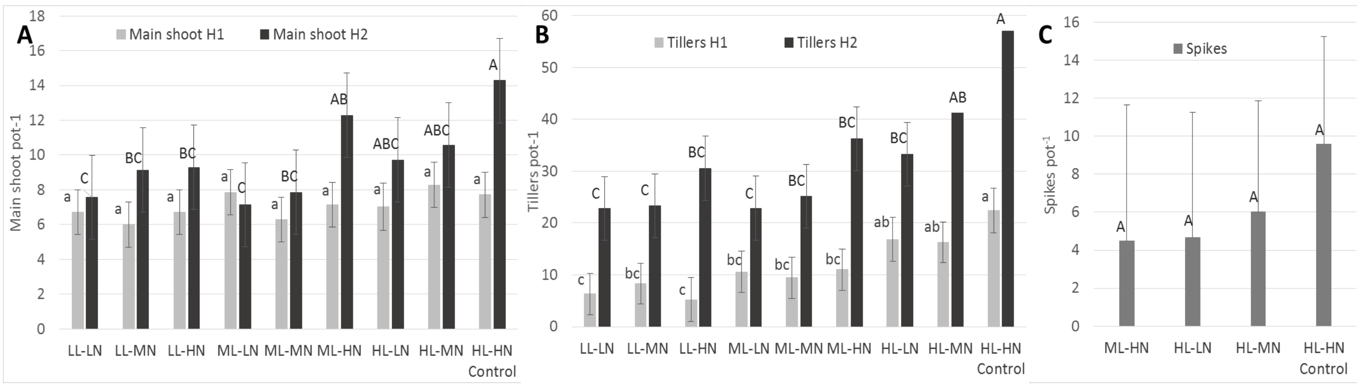

Figure 11. Number of main shoots (A) and (B) tillers per pot of *E. repens* when growing under different light and nutrient interactions and the number of spikes produced when growing under limiting light and nutrients (C) (Only produced in the second harvest). Please note the difference in scale in the Y-axes between shoots and tillers.

4.2 Cover crops competition.

For the second experiment, *E. repens* was grown in competition with cover crops to study the impact on its growth and the allocation of the resources. As presented in the ANOVA tables (Tables 4 and 5), in the first harvest red clover did not had any impact while ryegrass affected shoot dry weight, root fraction and number of tillers. In the second harvest both cover crops had a significant impact on *E. repens* dry weights, the number of shoots and tillers and the nitrogen percentage. On the other hand, while red clover did not have a significant impact on the value of dry weight fractions (except old rhizome fraction) ryegrass affected all except root fraction.

Table 4 *Results of the analysis of variance (ANOVA) showing the effect of cover crop treatments (Red Clover or Ryegrass) on* E. repens *dry weights and fractions. Bold letters indicate p-values smaller than 0.05*

		Root dw	New rhizome dw	Shoot dw	Old rhizome dw	Total dw	Roots fraction	New Rhizome fraction	Shoots fraction	Old Rhizome fraction
	DF	P	P	P	P	P	P	P	P	P
Harvest 1										
Red Clover	2	0.7865	0.3069	0.2868	0.4990	0.3971	0.3915	0.5170	0.1800	0.7120
Ryegrass	2	0.2422	0.0801	**0.0353**	**0.0481**	0.0620	**0.0205**	0.2499	0.0828	**0.0092**
Harvest 2										
Red Clover	2	**0.0016**	**<.0001**	**<.0001**	0.0035	**<.0001**	0.1901	0.2175	0.4539	**<.0001**
Ryegrass	2	**<.0001**	**<.0001**	**<.0001**	0.9093	**<.0001**	0.4429	**0.0444**	**0.0015**	**<.0001**

Table 5 *Results of the analysis of variance (ANOVA) showing the effect of cover crop treatments (Red Clover or Ryegrass) on* E. repens *shoot counting in both harvest and Carbon and Nitrogen percentage on the shoots of the second harvest. Bold letters indicate p-values smaller than 0.05.*

		Total	Main shoot	Tiller	Spikes	Tot-C % av ts	Tot-N % av ts
	DF	P	P	P	P	P	P
Harvest 1							
Red Clover	2	0.2497	0.6755	0.1176			
Ryegrass	2	**0.0148**	0.4155	**0.0085**			
Harvest 2							
Red Clover	2	**<.0001**	**0.0104**	**<.0001**	0.1554	0.0711	**0.0317**
Ryegrass	2	**<.0001**	**0.0006**	**<.0001**	0.0544	0.0839	**<.0001**

Reduction of *E. repens* biomass under cover crops.

In the first harvest, the impact of cover crops on *E. repens* growth was not significant, probably because the amount of light and resources on the ground were sufficient for both crops, so the competition process was not very intese at that point.

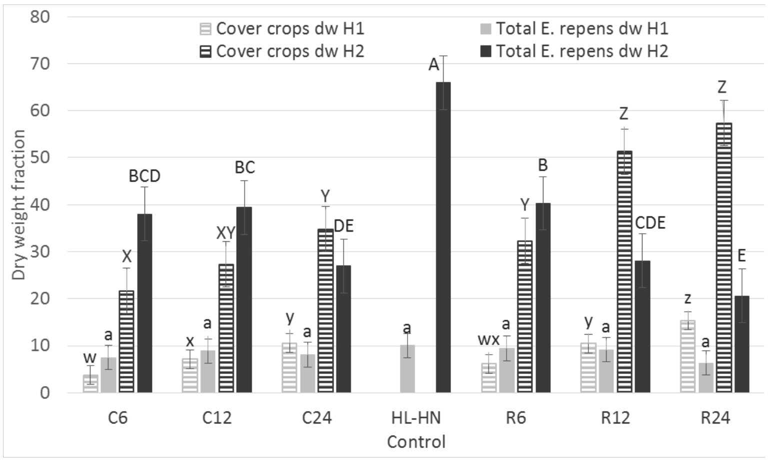

Figure 12. Total dry weight of *E. repens* and cover crops total dry weight, in harvest one and two. Error bars are 95% confidence interval and letters Tukey HSD test comparisons.

In the second harvest both species had enough time to grow and compete. The growth of *E. repens* under red clover or ryegrass was significantly decreased. This decrease was related to the growth of the cover crop itself, which was in direct correlation with its seeding density (Fig. 12). The total dry weight of *E. repens* was reduced by approximately 40 % in the low seeding density (C6, C12 and R6) and by almost 65 % in the high seeding density (C24 and R24). Growth decreased most when cultivated under ryegrass, with its high seeding density treatment (R24) resulting in the smallest *E. repens* total dry weight biomass. Even though ryegrass produced more biomass than red clover for the same seeding density the reduction of *E. repens* total biomass was not significant for equal number of seeds.

Allocation of the resources in *E. repens* when growing under cover crops.

Allocation changed compared to the control on ryegrass low and high seeding densities (R6 and R24). Resources shifted from above ground biomass (shoot) to below ground biomass (roots and new rhizome) and predominantly to the new rhizome formation (Table 4). On the other hand, the three red clover seeding densities or ryegrass medium density did not change the allocation pattern (Fig. 13 C and D).

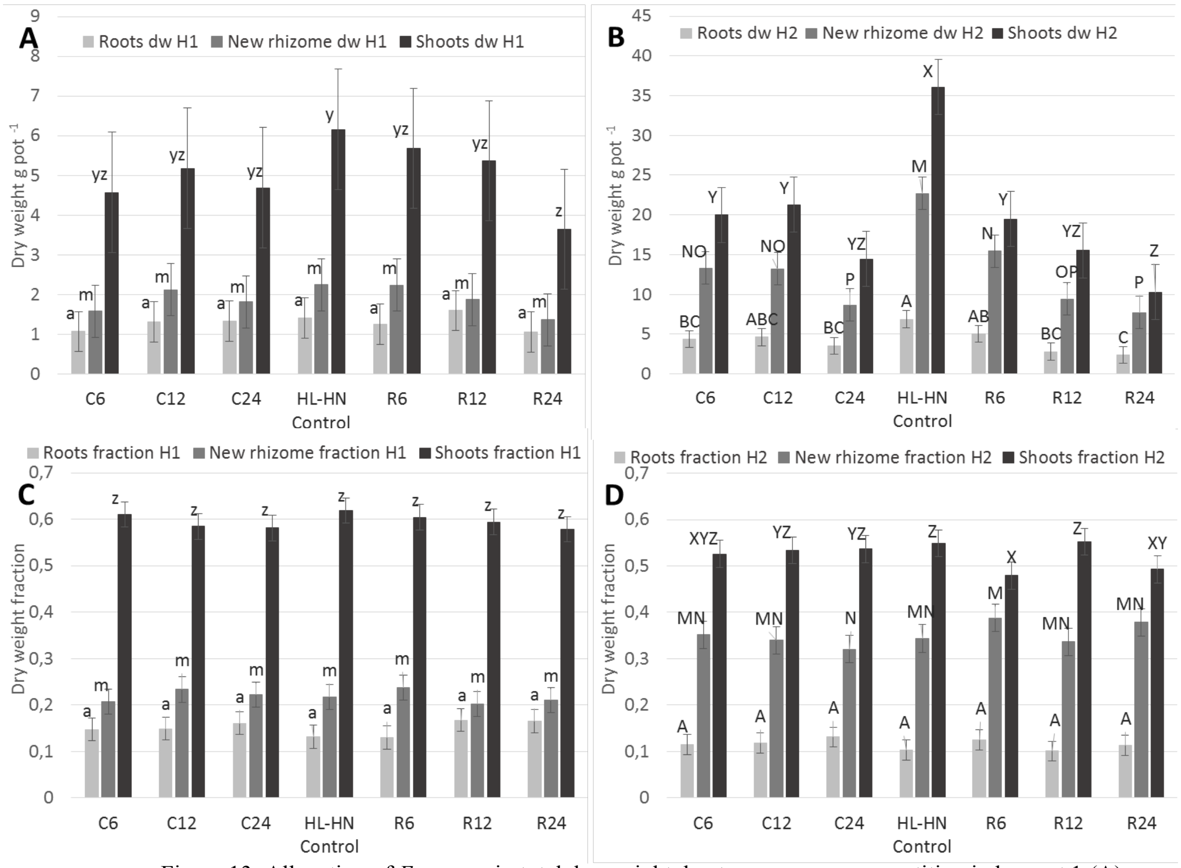

Figure 13. Allocation of *E. repens* in total dry weight due to cover crop competition in harvest 1 (A) and 2 (B). *E. repens* dry weight fractions due to cover crop competition in harvest 1 (C) and 2 (D).

Even thouhg carbon concentration was altered by both cover crops compared with control, red clover decreased it and ryegrass increased it (Fig. 14 A). Ryegrass reduced the nitrogen concentration on *E. repens* shoots compare to the control, while red clover only showed an effect on C12 (Fig. 14 B).

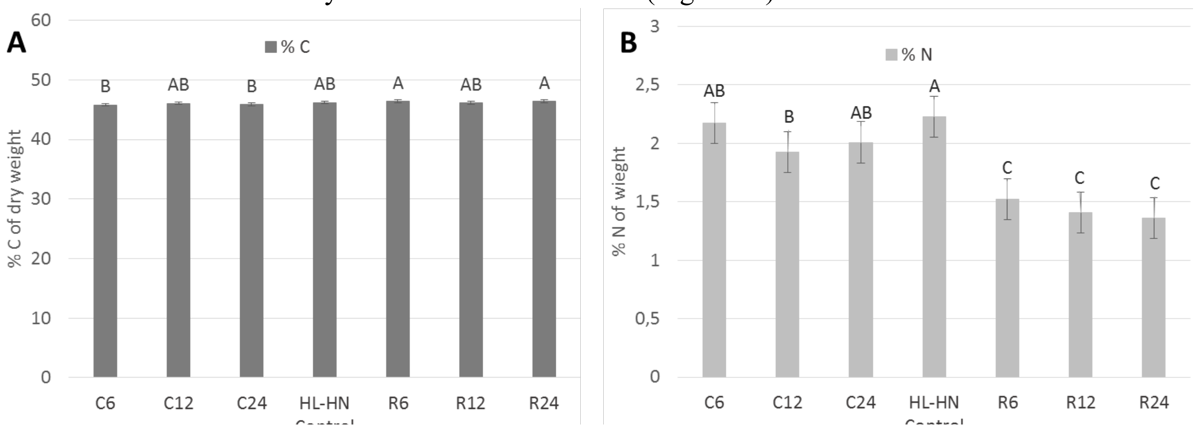

Figure 14. Carbon (C) (A) and Nitrogen (N) (B) concentrations in *E. repens* shoots, percentage in the dry weight, of the control and the red clover and ryegrass abiotic competition.

Morphological features of *E. repens* when growing under cover crops.

The total number of shoots produced by *E. repens* compared to the control when growing under cover crops was the same in the first harvest and decreased for the second one, remaining constant independently of the seeding (Fig. 15 A).

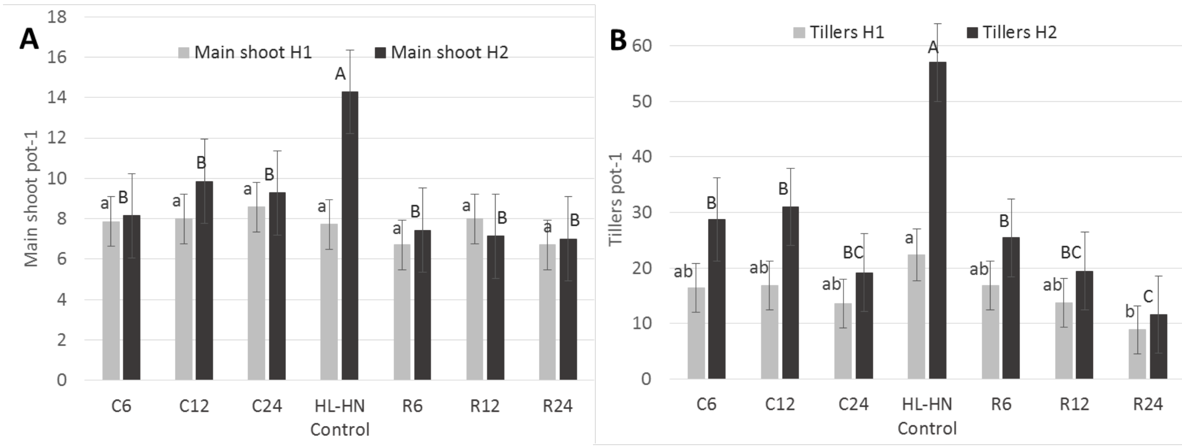

Figure 15. Number of shoots (A) and tillers (B) in harvest one and two.

Tillers also decreased on both cover crops in the second harvest. Ryegrass tillers showed a significant declining trend (Fig. 15 B). The proportion of tillers compared to main shoots decreased compared to the control for both cover crops.

Both cover crops tended to cause *E. repens* to present fewer spikes than the control by the end of harvest two (Fig. 16).

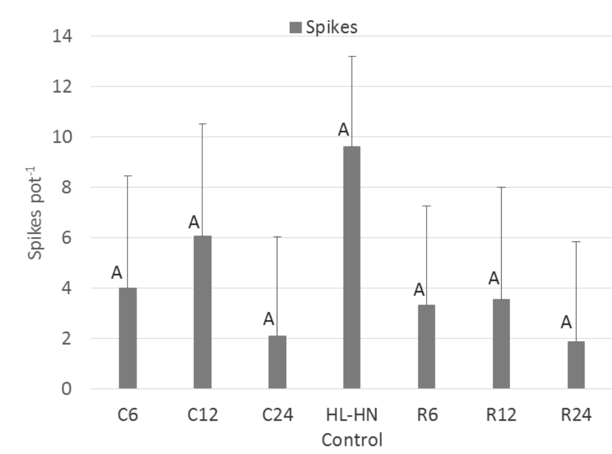

Figure 16. Number of spikes produced by E. repens under cover crops competition. Spikes were produced only in the second harvest.

5 Discussion

The first hypothesis that artificially limiting light and/or nutrients reduce the total dry weight of *E. repens*, was confirmed. Root, rhizome and shoot dry weights were negatively affected by reduced light, whereas only shoot dry weight was significantly affected by decreasing nutrient availability (Table 2, Fig. 7 A and B). The biomass reduction due to limited light and/or nutrients was greater compared to the control in the second harvest as *E. repens* plants had been exposed to limited resources for a longer period of time (Fig. 6).

The second hypothesis, which stated that limiting light intensity would shift the allocation of resources towards the above ground fraction was also confirmed (Fig. 8 C and D). Likewise, in accordance with the third hypothesis, limiting nutrient availability shifted the allocation of resources from shoots to the below ground, but only in the second harvest (Fig. 9 D).

In the first harvest, limiting light had a greater impact on *E. repens* allocation than nutrient availability (Fig. 9 C). As light increased *E. repens* produced more biomass, and the allocation of resources shifted from shoots to the below ground (Fig. 8 A and C). Nutrient availability had a smaller impact on the amount of biomass produced (Fig. 9 A and C), the allocation did not change at any concentration level. This is probably caused because the plants had enough nutrients either stored in old rhizomes or in the soils for it to be a deficient resource. Results indicate that at the first growth stages, light availability is important. Under lower light, *E. repens* directed the resources to build the root system to anchor the plant to the ground and maximize absorption of nutrients as well as to sprout new shoots and therefore increase photosynthesis. To increase the amount of light received *E. repens* produced fewer but longer shoots (etiolation). This results are consistent with the findings of Skuterud (1984), that reduced light intensity increased stem length. Under higher light intensity, the rhizome system development was greater.

Later in the growing season, nutrients became a limiting resource especially at higher light intensities (Fig. 9 B). In the second harvest light intensity followed the same pattern as in the first (Fig. 8 B and D) but lower nutrient availability deceased total biomass and changed the allocation pattern from above ground to roots and rhizomes (Fig. 9 B and D). This is comparable to McIntyre's (1965) experiment on *E. repens* where a reduced amount of nitrogen supply decreased shoot dry weight without reducing roots or rhizomes dry weights (Fig 9 D). By creating a more extensive below ground system, *E. repens* is able to access a larger portion of the soil and absorb more nutrients in accordance to Kleijn and Van Groenendael (1999) and his experiments on rhizome expansion at different productivity levels. Old rhizomes dry weight were lower in the first harvest than in the second, due to a refill during the later growth period. As old rhizome biomass was higher in the low nutrient treatments, it may be more cost-efficient for *E. repens* to maintain old rhizome than growing new ones. *Elymus repens* only formed spikes in high light, when the formation of rhizome biomass was also greatest (Fig. 11 C), in contradiction to Williams (1973) who found that plants with lower rhizome weight tended to have the higher weight of spikes.

According to Keddy and Keddy, (2012) competition is the negative effect on a certain plant caused by the presence of neighbors which reduce the availability of resources, thus having an important effect upon growth and reproduction. Tilman (1988) defines competition as the sum of exploitative competition and allelopathic competition. The effect of competition against E. repens has been shown by several authors as Dyke and Barnard (1976), Loeppky and Derksen (1994) or Melander *et al.* (2012). In the second experiment, red clover and ryegrass were used to test the effect of competition on allocation of *E. repens*. Due to the complexity of biotic competition the patterns were not as clear as in the first experiment. Regardless of the cover crop type, a higher seeding density reduced *E. repens* total biomass more than a lower seeding density (Fig. 12). As in the first experiment the reduction of total *E. repens* total biomass was related to the competition period as the reduction was greater in the second harvest (Fig. 13 B). Red clover reduced total biomass of *E. repens* as expected (Fig. 12). However, the last hypothesis that red clover would compete strongly enough for light to force *E. repens* to allocate resources to shoots, was not confirmed. Despite the dense red clover canopy, *E. repens* fractions did not change compared to the control at any seeding density (Table 4, Fig. 13 C and D). At the highest seeding density (C24) red clover did however lower the number of *E. repens* shoots compared to control and the other red clover treatments, potentially indicating a higher amount of light competition.

Even though both cover crops reduced total biomass, ryegrass had a larger effect on the growth of *E. repens* causing a greater reduction in total biomass and a change in the allocation pattern (Fig. 13 D). Ryegrass forced *E. repens* to change the allocation from the above ground to the below ground in R6 and R24 (Fig. 14 D). Thus, ryegrass, seemed to primarily compete with *E. repens* for nutrients, as stated in the last hypothesis. There have been several studies proving the competitive effect of ryegrass as Jeangros and Nösberger (1990) in their experiment on *Rumex obtusifolius* L. or López *et al.* (2013) on *Bromus valdivianus* Phil. The reduction of nitrogen percentage in the leaves compared to the control (Fig. 14 B) indicates that nitrogen was lower in these treatments due to competition.

Here, treatment R12 (12 seeds per box) did not change the allocation pattern from shoots to rhizomes as expected even though R6 and R24 did. Ryegrass produced a similar amount of total biomass at R12 and R24 although R24 had twice as many plants as R12. One possibility is that at R12, ryegrass grew a denser canopy shadowing *E. repens* more than at R24 while absorbing the same proportion of nutrients, resulting in a similar neutral state as red clover. In R12 it may have been primarily an interspecific competition whereas in R24 the intraspecific competition may have been higher. The allocation pattern was non-linearly related with ryegrass biomass.

E. repens produced spikes when growing under both cover crops and all seeding densities (Fig. 16). Even under competitive conditions, *E. repens* directed resources for sexual reproduction. In the first experiment resources at lower light intensities were directed to the production of tillers as in the field experiment of Westra and Wyse (1981) where a low spike production appeared to be related to an increase in tiller production.

The interest of this experiment can be related to control efforts and further research. Limiting resources and cover crops did reduce the total amount of *E. repens*. The change in the allocation pattern of red clover and ryegrass in controlling *E. repens* depends on the use or not of other control methods. For example effective mowing may benefit from a lower shoot fraction, since each leave counts for photosynthesis, but it may cause a less effective mowing since there is less aerial biomass to cut. Future research can benefit from this experiment when aiming at a more accurate estimation of the correlation between the number above ground structures and the below ground organs.

In further experiments, I would suggest to repeat this experiment under natural conditions, to study different seeding densities and the optimal time of cover crop introduction in the soil to make the maximum growth of the cover crops coincide with the non-compensation point of the weed. It would be interesting to study the presumable continuous reduction of *E. repens* total dry weight, *E. repens* competitive strategies and its relation to different seeding densities in several growing seasons.

Conclusions

In conclusion, *E. repens* allocation patterns are altered by environment and competition. Reduced light intensity increased shoot biomass fraction and decreased the number of tillers and spikes. Limited nutrient availability increased the below ground fraction (roots and rhizomes) and decreased the number of tillers. As anticipated, cover crop competition reduced *E. repens* total biomass, number of tillers and spikes showed the ability to change the allocation pattern. Ryegrass competed for nutrients shifting *E. repens* allocation towards rhizomes. However, neither red clover nor ryegrass seemed to compete strongly enough for light since there was no change in the allocation pattern towards the shoot biomass fraction.

Regardless of whether the reduction in the different biomass fractions can be useful or not, cover crops did reduce the total biomass of *E. repens*. A combination of other control methods and the under-sowing of cover crops in the crop rotation every year may significantly reduce the presence of *E. repens* in arable land.

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