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Investigation of the effect of different leguminous cover crops on the nitrogen use efficiency of spring barley

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Contents

List of abbreviations and acronyms

N: Nitrogen, P: Phosphate, K: Potassium, NFC: Nitrogen Fixing Crop, GM: Green Manure, NUE: Nitrogen Use Efficiency, GS: Growth Stage, BNU: Biomass Use Efficiency, DAS: Days after sowing, L: Low, H: High, SMN: Soil Mineral Nitrogen, SB: Spring barley, Var: variety, TBI: Tea Bag Index

Abstract

The research focus was to investigate the effect of different Nitrogen Fixing Crops (NFC) used as green manure (GM) on the Nitrogen Use Efficiency (NUE) of spring barley (SB) (*Hordeum Vulgare* var Westminster), with different N-fertiliser treatments, in Scotland cold-temperate climatic conditions. Different GM's treatments were grown during a year, then, SB was sown in it. A low rate of N-fertiliser was applied to half of the SB plots to study the effect of mixed N sources (NFC used as GM and mineral N-fertiliser) on NUE. The N fluctuation in soil, the soil fauna activity and the crop biomass production according to the different treatments were assessed. The analysis of the decomposition rate, the N content of the SB crops and, thus, the calculation of the NUE could not be carried out due to technical and time issues. To replace temporarily the NUE, the amount of biomass created per unit of N available was calculated (BNU=BiomassGS60/(SMN GS0 $\pm$Nfertiliser)) and called Biomass N Use (BNU), with BiomassGS60 being the SB biomass at anthesis, SMN GS0 being the soil mineral N content at SB sowing and N-fertiliser being the N applied as fertiliser.
The NFC treatments enhanced the soil mineral N content and triggered the soil fauna activity in comparison with the controls but had no noticeable effect on the SB biomass production. The addition of N-fertiliser has shown to increase the soil mineral N content, the biomass of the SB, and supposedly will improve the grain yield. The BNU of SB varied accordingly to the previous NFC and N-fertiliser treatments. The addition of N-fertiliser drastically decreased the BNU while it improved of 15% the crop biomass production. While the BNU of the NFC treatments with addition of N-fertiliser seemed all similar, without N-fertiliser they have shown interesting differences; with control (ryegrass) being one of the highest BNU, alongside with lupin, peas, mixture B and white clover, which could mean that some legumes could be as effective as grass at removing N from the soil while fixing N from the atmosphere. However, the relevance of BNU calculation is questionable because there is no evidence that it might represent reliably the NUE.

1. Introduction

Today, the main source of nitrogen (N) applied in fields in agriculture comes from inorganic synthesised N-fertiliser, followed by animal manure (Hirel et al, 2011); but other sources exist such as biological N fixation, recycling of N from crop residues and atmospheric deposition (Smil, 1999 cited in Anbessa & Juskiw, 2012). From 50 to 75 % of this applied N is lost through leaching process (Hodge et al, 2000; Asghari & Cavagnaro, 2011, Hirel et al, 2011). N leaching is responsible for many environmental issues, especially water contamination by nitrate. Nitrate contaminated water is not drinkable anymore and in high concentration brings serious risks for animal's health, which includes humans', and lead to eutrophication of aquatic ecosystems and destruction of the environment (London, 2005; Beman et al, 2005; Camarguo & Alonso, 2006). The process from which most of the production of synthetic N-fertiliser is derived, called Haber-Bosch process, is very polluting because it releases a considerable amount of CO_2 and has a high fossil fuel energy cost (Andrews & Lea, 2003).

Most of the studies on green manure (GM) focus on the N supply because it is hugely needed by the plant and it is often the limiting element for growth and yield of the crops (Andrews & Lea, 2003). The use of cover crop and GM could prevent the N leaching phenomenon (Tonitto et al, 2005) and be a much more sustainable alternative to N-fertilisers. It aims to capture the excess of inorganic N in the soil before it is lost, to subsequently return it to the following crop. Legume-based GMs, able to do Biological N fixation, even add N to the soil by converting atmospheric N into a form that is directly available for plant uptake.

Cover crop and GM bring several benefits for crop production and participate to soil conservation. Cover crop can be defined as any non-cash crop, grown to improve soil structure, fertility and consistency (Fageria, 2005). GM is created incorporating fresh cover crops into the soil to increase the level of nutrients and organic matter in the soil (Fageria, 2005) and thus improving the subsequent cash crop grown in it.

According to Hendrickson (2009) the soil quality benefits of cover crop include the protection against soil erosion, building and maintaining both active and stable organic matter, improving soil structure and tilth, improving the capillary action, or upward movement of water, within soils, increasing the biological activity in soils, the fracturing of hardpan by deeply rooting cover crops and the addition of N to the soil by legume cover crops.

Cover crop species are very numerous and the success of their use depends on a good choice of the species, an adapted management, a good timing and planning (Thorup-Kristensen et al, 2003). A careful synchronization of N mineralisation with crop growth is necessary especially in low inputs system (organic farming), in order to sustain high

production and limit environmental impacts (N leaching, greenhouse gas emissions) (Borgen et al, 2011).
The N mineralisation and decomposition rate of the cover crop are affected by several factors. These include the quality of the GM (N content, C:N ratio, lignin, polyphenol and other organic compounds content, the plant age,…), the environmental factors (temperature, moisture, soil microbial activity,…), management factors (method of incorporation, size of particles,…) and the soil characteristics (Yadvinder-Singh et al, 1992; Thorup-Kristensen et al, 2003).
It is commonly considered that healthy soils have an important soil's life activity and by extension an important soil fauna feeding activity. The bait-lamina stick method (Von Törne, 1990), by giving an idea of the feeding activity of the soil fauna, is an assessment of the potential influence of different agricultural practices on soil's health.

While the effect of cover crops to reduce N loss by leaching is quite well known, their effect on the N supply for the succeeding crop is a bit less clear (Thorup-Kristensen et al, 2003). It is hard to assess if any N-fertiliser is needed in addition to cover crop, mainly because it depends on many parameters, and also on goals set. More research is needed on the management and synchronization of release and demand of N, especially because it varies with the cover crop species, the environment and climatic conditions and the management practices (Thorup-Kristensen et al, 2003).
Generally, GM/cover crops are sown in autumn and ploughed in spring, before sowing the cash crop, while, due to climatic conditions, this is often not possible in Scotland. GM needs to grow a whole season, thus taking the place of the cash crop. To replace a cash crop by GM is expensive, which makes many farmers reluctant to use it; therefore, their benefits have to compensate for the losses. More researches are needed to make the use of GM the most effective possible. GM, as being an option that could be more sustainable than mineral or animal fertilisers, has to be explored in depth.

Increasing the Nitrogen Use Efficiency (NUE) of the crops could help to reduce the losses of N and thus the sustainability of agricultural systems. The NUE is generally assessed as the amount of grain yield per unit of N supplied (Moll et al. 1982; Good et al. 2004; Fageria et al. 2008) but can be calculated otherwise. An enhanced NUE results in a higher efficiency at catching up the N from the soil by the plants.
For this experiment, different cover crops (legumes alone or in mixtures) were grown during one year, from spring 2016 to spring 2017, when they got ploughed in. SB was sown in it and a range of measurements was taken on soil and SB during its growth to assess their N fluctuation, the N mineralisation of the different cover crops/mixtures, their associated soil fauna activity and the NUE of the subsequent crop (spring barley).

The research focus was to investigate the effect of different leguminous cover crops on the NUE of spring barley (SB), with different N-fertiliser treatments, in Scotland cold-temperate climatic conditions.
The aims of the research project were:

- To assess the effects of the preceding N-fixing crops on soil mineral N (SMN) status, decomposition rates and soil fauna activity.
- To assess the pre-anthesis NUE of SB
- To assess the effect of mixed N sources (NFC and N-fertiliser) on SB in term of yield and NUE.
- To assess the agronomic and economic performance of SB growth when succeeding N-fixing cover crops, with and without added N-fertiliser.

The hypothesis were that 1) The different cover crops/mixtures will be mineralised at different rates, 2) These rates will be related to the soil life community, 3) The soil life activity and mineralisation rate will affect the NUE, 4) the NUE will vary according to the preceding NFC treatments and 5) according to the N-fertiliser treatment and 6) that using NFC and N-fertiliser as mixed N sources can maintain high yield while lowering the environmental costs.

This report presents the assessment of the effect of the preceding N-fixing crops and N-fertiliser treatments on the soil fauna activity, on the soil mineral N content, on the SB biomass production and an estimated version of the NUE of SB, called Biomass N Use (BNU).

2. Methodology

2.1. Site, experimental design and crop management

The experimental field was located in Saphock farm, Aberdeenshire, Scotland. This field had been used to grow conventional cereals for the two years preceding our experiment (2014 and 2015). For more details about the historic of Saphock farm, see **appendix 1**.
Saphock farm's soil was ranked as class 3 out of 7 on the national land capability scale for agriculture, 1 being the best sort of soil (Bernie et al, 2010). It is a normal land class to use for arable farming in the North-East of Scotland (there is no Class 1 or 2 land in this area).

Different N-fixing cover crops (see **table 1**) were grown for a year as GM to subsequently grow SB (Hordeum vulgare var Westminster) in it. The legumes were grown approximately a year (from mid May 2016 to the end of March 2017), then were incorporated into the soil in spring 2017. One week later, in the beginning of April 2017, the SB was sown. The time between ploughing the NFC and sowing the SB was decided taking into account the synchronisation of the NFC N release and the barley needs and the climatic conditions of the moment influencing strongly the decomposition rate of the NFC.

Abreviation	**English name**	**Latin name**	**Variety**
LU	Lucerne/alfalfa	*Medicago saliva* L.	Neptune
BM	Black Medic	*Medicago lupulina*	Virgo pajbjerg
BE	Beans	*Vicia faba*	Fuego
WV	Vetch	*Vicia sativa*	Jose
LN	Lupin	*Lupinus angustifolius*	Iris
PE	Peas	*Pisum sativum*	Zero4
RC	Red clover	*Trifolium pratense*	Merula
WC	White clover	*Trifolium repens*	Aberpearl
CC	Crimson clover	*Trifolium incarnatum*	Pier

Table 1: NFC 2016 legumes variety

The NFC grown consisted in 14 different legumes treatments: 9 straight and 5 mixtures, plus 2 controls. The NFC field trial layout is presented in **fig1**. Each NFC treatment was sown in double-plots of 4m by 10m and replicated 3 times, and each control was sown in single-plots of 2m by 10m and replicated 6 times. The overall surface of each treatment and control was the same. The field size was 30 by 60m with each block being 10m wide by 60 m long. Each block began and ended with controls/guards: GC and RG.

The 9 straight treatments were Lucerne (LU), Black medic (BM), Beans (BE), Vetch (WV), Lupin (LN), Peas (PE), Red clover (RC), White clover (WC) and Crimson clover (CC). The different mixtures were Mixture A: RC, LU and BM; Mixture B: RC, WC and CC; Mixture C: WC, PE and WV; Mixture D: WC, BE and WV and Mixture E: WC, BM and WV. The controls were a grass/clover (GC) mix and ryegrass (RG). The seed rates (**appendix 2** for the straight and **appendix 3** for the mixtures) were calculated depending on the weight of each sort of seeds to have the same amount of seeds sown per plot.

NFCs were crushed before being incorporated into the soil. The field trial layout after sowing of the SB is presented in **fig2**.

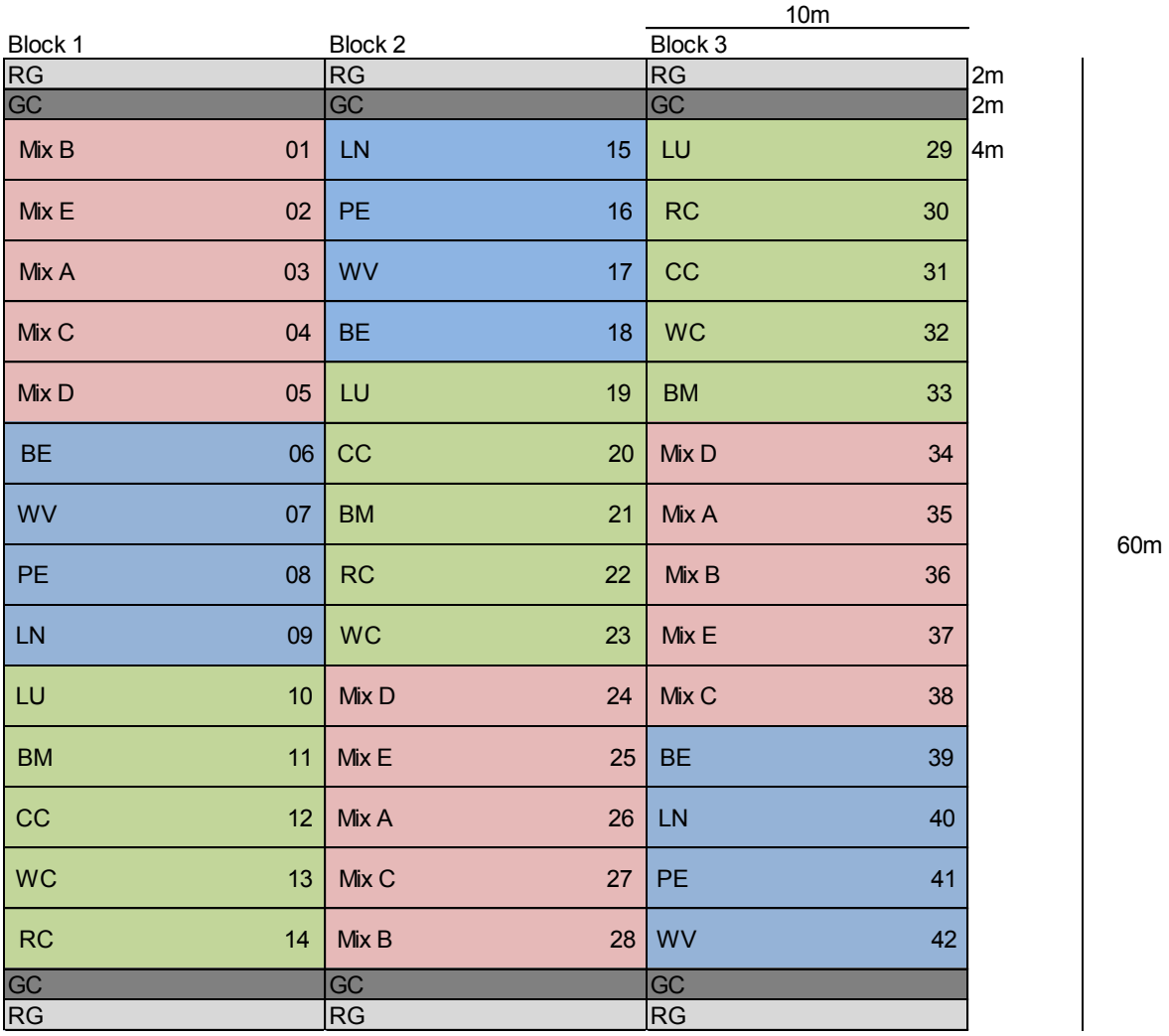

Fig1: Nitrogen Fixing Crop (NFC) 2016 field trial layout. The mixtures are shown in red, the grain legumes in blue and the clovers in green. GC: Grass/clover; RG: Ryegrass; Mix B (mixture B)= red clover, white clover and crimson clover; Mix E= white clover, black medic and vetch; Mix A= red clover, black medic and lucerne; Mix C= white clover, vetch and lupin; Mix D= white clover, vetch and beans; BE: beans; WV: vetch; PE: peas; LN: lupin; LU: lucerne; BM: black medic; CC: crimson clover; WC: white clover; RC: red clover.

				10m			
Block 1		Block 2		Block 3			
Barley	RG L	Barley	RG L	Barley	RG L	2 m	
Barley	GC L	Barley	GC L	Barley	GC L	2 m	
Barley	01 L	Barley	15 L	Barley	29 L	4 m	
Barley	01 H	Barley	15 H	Barley	29 H		
Barley	02 L	Barley	16 L	Barley	30 L		
Barley	02 H	Barley	16 H	Barley	30 H		
Barley	03 L	Barley	17 L	Barley	31 L		
Barley	03 H	Barley	17 H	Barley	31 H		
Barley	04 L	Barley	18 L	Barley	32 L		
Barley	04 H	Barley	18 H	Barley	32 H		
Barley	05 L	Barley	19 L	Barley	33 L		
Barley	05 H	Barley	19 H	Barley	33 H		
Barley	06 L	Barley	20 L	Barley	34 L		
Barley	06H	Barley	20 H	Barley	34 H		
Barley	07 L	Barley	21 L	Barley	35 L		
Barley	07 H	Barley	21 H	Barley	35 H		
Barley	08 L	Barley	22 L	Barley	36 L		60 m
Barley	08 H	Barley	22 H	Barley	36 H		
Barley	09 L	Barley	23 L	Barley	37 L		
Barley	09 H	Barley	23 H	Barley	37 H		
Barley	10 L	Barley	24 L	Barley	38 L		
Barley	10 H	Barley	24 H	Barley	38 H		
Barley	11 L	Barley	25 L	Barley	39 L		
Barley	11 H	Barley	25 H	Barley	39 H		
Barley	12 L	Barley	26 L	Barley	40 L		
Barley	12 H	Barley	26 H	Barley	40 H		
Barley	13 L	Barley	27 L	Barley	41 L		
Barley	13 H	Barley	27 H	Barley	41 H		
Barley	14 L	Barley	28 L	Barley	42 L		
Barley	14 H	Barley	28 H	Barley	42 H		
Barley	GC H	Barley	GC H	Barley	GC H		
Barley	RG H	Barley	RG H	Barley	RG H		

Fig2: Spring barley 2017 field trial layout. The 42 plots have been split in two and half on them have been applied 60 kg.ha^{-1} of N-fertiliser. The colours show the previous NFCs that have been ploughed in (mixtures in red, grain legumes in blue and clovers in green). L: no N-fertiliser treatment; H: +60 kg.ha^{-1} N-fertiliser treatment; GC: Grass/clover; RG: Ryegrass

In 2016, during the legumes growth, copper and manganese were applied as a fertiliser into the field after being found to be in deficiency. In 2017, every plot of barley received P-K fertiliser (0-24-24) at a rate of 75kg.ha^{-1} the first week of May. An herbicide was applied in late spring 2017, during the SB growth. From spring 2017 to summer 2017, while the barley was growing a range of measurements was taken on the barley and the soil.

In the end of May, half of the plots and guards/control were applied a rate of N-fertiliser, 60kg/ha^{-1}, which was ½ of recommendation, to study the effect of mixed N sources (cover

crop and N mineral fertiliser) on NUE. The recommended rate was established by SAC consulting, The Farm Management Handbook 2016/17. The N-fertiliser was applied 7 weeks after barley sowing. As a convention throughout this study, the treatment with no N-fertiliser was called Low (L) and the treatment with addition of 60kg.ha^{-1} of N-fertiliser was called High (H).

See **appendix 4** to know more about the student's role.

2.2. Sampling & measurements

Samples and measurements were taken along the NFC and SB growth to provide detailed N evolution according to the different treatments.

2.2.1. Soil fauna feeding activity

The bait-lamina stick method was first described by Von Törne (1990) to estimate the soil fauna feeding activity. It consisted in using a bait-lamina stick (**appendix 5**) with 16 holes (2 mm of diameter) spread on 8 cm (one hole every 0.5 cm) and fill the holes with feed substrate. While it can vary, here, the substrate (Terra Protecta GmbH) consisted of 70% of cellulose (micro-granular), 27% of bran flakes (<500 mm), and 3% activated carbon as described by Eisenhauer et al, 2014. This experiment was carried out during the SB growth, just after SB's growth stage 30. They were 8 sticks buried per plot and 3 replicates per previous NFC treatment tested (Mix A, Mix B, LU, BM, CC, WC and CNTR). This assessment was realised during the SB growth. Only previous NFC treatments that were clovers (Mix A and B being only clovers mixtures) and received no N-fertiliser (L) were tested. It was not realised for the controls RG and GC. Instead, the control (CNTR) used here was a field of conventional SB for the past 2 years with no previous NFC/GM treatments, normal fertiliser application (i.e. 120 kg.ha^{-1} according to SAC consulting, The Farm Management Handbook 2016/17) and stubble/fallow over winter. The bait sticks were removed after 9 days into the soils and were analysed. The consumption rate on the stick was a measure of the feeding activity of soil invertebrates. The feeding activity was recorded for each hole; "0" when the substrate was intact, "0.5" when the substrate was perforated partially, and "1" when the substrate was fully removed. Microorganism and abiotic decay were considered as negligible (Helling et al. 1998, Hamel et al. 2007). The average feeding activity of the soil fauna was assessed according to the depth and to the NFC treatment separately.

2.2.2. Spring barley (SB) biomass production

It was decided to sample the SB biomass at Growth Stage 30 (GS30), which correspond to the beginning of the stem elongation with the ear at 1 cm (**appendix 6**), and at Growth Stage 60 (GS60) which correspond to the full emergence of the ear (**appendix 7**), just before the starting of flowering. For more information about the SB growth stages, see **appendix 8**. At GS30 and GS60 aboveground crop samples were taken from a 0.5x0.5m random quadrat. The samples were separated into crop and weeds. Samples were dried at 80°C, weighed and recorded.

2.2.3. Evolution of the soil mineral N (SMN) content

Soil samples were taken in each plot to assess the soil mineral N (SMN) content at 5 different stages (see the timeline in **fig3**). For ease of understanding, they were referred as Days After Sowing (DAS) the SB. The different soil samples were taken at DAS-127, DAS-78, DAS0, DAS+67 and DAS+103.

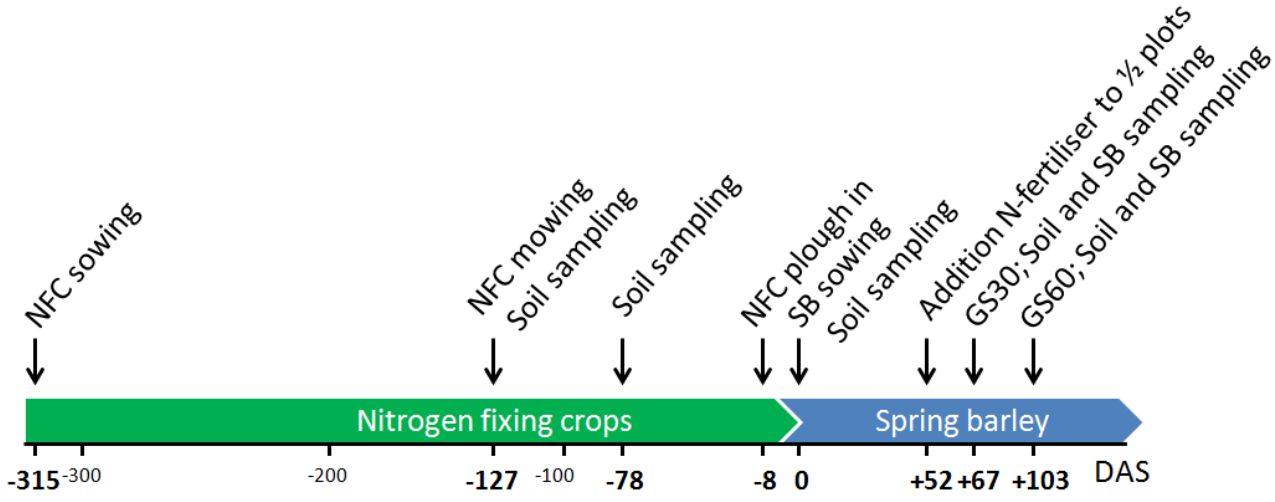

Fig3: Experimental timeline. Time was expressed in Days After Sowing (DAS) the spring barley (SB). NFC: Nitrogen fixing crops; GS: growth stage of the barley.

Three soil cores (2-4 cm diameter) were taken per plot, split into two depth fractions (0-15 cm, 15-30 cm) and pooled to give 2 samples of 1kg each per plot. Each sample was well mixed, and sub-samples were performed. A dilution by 1/5 of soil in KCl was made for N analysis. The N was quantified under the form of ammonium (NH4-N) and nitrate (NO3-N). The N analysis was performed by SRUC Edinburgh, with a device called Skalar, San++ continuous flow colorimetric analyser. The SMN content was determined under the form of ammonium and nitrate.

To assess the percentage of soil humidity, approximately 30g of fresh humid soil was weighted, then oven-dried at 105°C for 24h and weighted again. This measure was used into the calculation of the quantity of N per hectare.

2.3. Statistical analysis, calculations

The SMN content was estimated as the sum of ammonium (NH_4^+) and nitrate (NO_3^-). The amount of NH_4^+ (or NO_3^-) in kg.ha^{-1}, called yNH_4^+ (or yNO_3^-), was calculated with the following formula:

$$yNH4 + (or\ NO3-) = \frac{50 * \text{BulkDensity} * \text{NH4(orNO3)ppm}}{100 - \%water} * \frac{100 - \%\text{stones}}{100} * \text{CoreDepth}$$

For each sample, the sum of yNH_4^+ and yNO_3^- gave the SMN content.

If the crop N content could have been analysed on time, a NUE would have been estimated as the N content of the crop at GS60 per the N supplied (Ns) to the crops:

$$\text{NUE} = \frac{\text{total N GS60}}{\text{Ns}}$$

with Ns being the SMN at GS0 (SB sowing) plus the N applied as a fertiliser plus the residual SMN (rSMN) measured in the soil post-harvest:

$$Ns = \text{GS0 SMN} + \text{Nfertiliser} + \text{rSMN post harvest}$$

Since it was not possible to estimate the NUE due to missing time and data, an early version of the NUE was estimated by calculating the amount of biomass created at GS60 per kg of N present in the soil at GS0.The 60 kg.ha^{-1} of N-fertiliser added in the H N-fertiliser treatment were taken into consideration into the calculation. This measure was called the Biomass Nitrogen Use (BNU). It was calculated as the SB biomass at anthesis (GS60) (in kg.ha^{-1}) per unit of SMN content at SB sowing (GS0) (in kg.ha^{-1}) more or less the N applied as fertiliser (in kg.ha^{-1}):

$$BNU = \frac{\text{BiomassGS60}}{\text{SMN GS0} \pm \text{Nfertiliser}}$$

The statistic analyses were carried out with the software Genstat.

Soil fauna feeding activity was compared between treatments using an ANOVA with the feeding activity as Y variate, the NFC treatment x Depth as treatment structure and the replicates as block structures.

The soil mineral N content was compared between treatments using an ANOVA with the SMN in $kg.ha^{-1}$ as Y variate, with the NFC treatment x N-fertiliser treatment x time (in days after sowing DAS) as treatment structure and with the replicates/ time (in DAS) as block structure.

The crop biomass production was compared between treatments using an ANOVA with the crop biomass in $kg.ha^{-1}$ as Y variate, with the NFC treatment x N-fertiliser treatment x time (GS30 and GS60) as treatment structure and with the replicates/time (GS30 and GS60) as block structure.

To look for a correlation between the SB biomass production and the SMN content, Spearman's test was performed with the crop biomass at GS60 as the response variate and the SMN content at DAS-78, DAS+0 and DAS+68 as explanatory variate.

Fischer's LSD test was performed on the fauna feeding activity and the SMN content to show the significance between the values obtained according to the different treatments.

3. Results

3.1. Soil fauna feeding activity

The depth was shown to have a significant effect on the soil fauna feeding activity ($F_{15,3049}$=11,33; $p < 0,001$). The feeding activity was higher close to the surface and decreased with the depth (**fig4**) with the highest activity (0,81) at 1,5 cm deep and the lowest activity (0,50) at 8 cm deep.

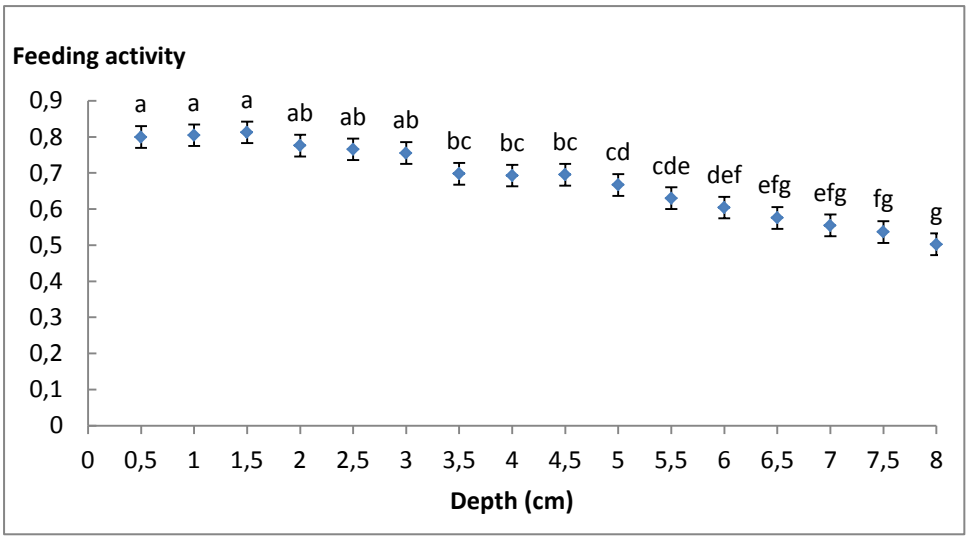

Fig4: Average soil fauna feeding activity according to the depth. Average feeding activities with the same letter above each dot are not significantly different (Fisher's LSD test) (P<0,001). The vertical bars represent the standard error of the difference between means.

Soil fauna feeding activity was found to be significantly different according to the previous NFC treatment ($F_{7,3057}$=27,68; p <0,001). The control (CNTR) shows the lowest fauna feeding activity with 0,44 followed by CC, WC, Mix B, LU, BM, RC and Mix A, respectively 0,64; 0,67; 0,69; 0,70; 0,73; 0,77 and 0,81(**fig5**). All the NFC treatments performed a significantly higher activity from the control (CNTR). Different level of significance were shown between the NFC treatments; Mix A and RC were significantly higher from LU, Mix B, WC and CC, in addition than from the control.

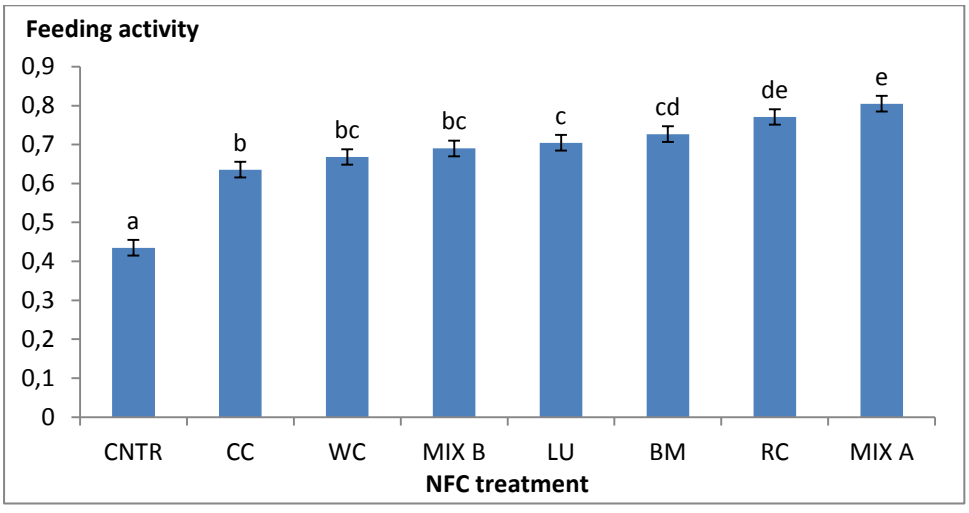

Fig5: Average soil fauna feeding activity according to the previous NFC treatments. CNTR: control (field of spring barley with no cover/fallow over winter); CC: crimson clover; WC: white clover; Mix B: red clover, white clover and crimson clover; BM: black medic; LU: lucerne; RC: red clover; Mix A: red clover, lucerne and black medic. Average feeding activities with the same letter above each column are not significantly different (Fisher's LSD test) (P<0,001). The vertical bars represent the standard error of the difference between means.

3.2. Evolution of the soil mineral N (SMN) content

The evolution of the average SMN content for the NFC treatments with (H) and without (L) addition of 60kg.ha^{-1} of N-fertiliser and for the control (RG (L)) showed different profiles (**fig6**). The average SMN for the NFC treatments was the same between the L and H N-fertiliser at DAS-127, DAS-78 and DAS0 because until then they have had the same N-fertiliser treatment (i.e. none). The N-fertiliser was only added between DAS0 (GS0) and DAS+67 (GS30) to the H, precisely at DAS+52. The N-fertiliser treatment (L and H) showed significant difference for stage DAS+67 ($F_{1;92}$=60,85; p <0,001). The overall SMN content of the control (RG L) was lower than for the NFC treatments. RG SMN content seemed to be lower at DAS+67 than at DAS+103 but this result were not significantly different from the NFC treatment that received not N-fertiliser (L).

At DAS+103, Control, L and H treatments did not show significantly different SMN content (respectively 10,08 7,96 and 10,64 kg of N.ha^{-1}).

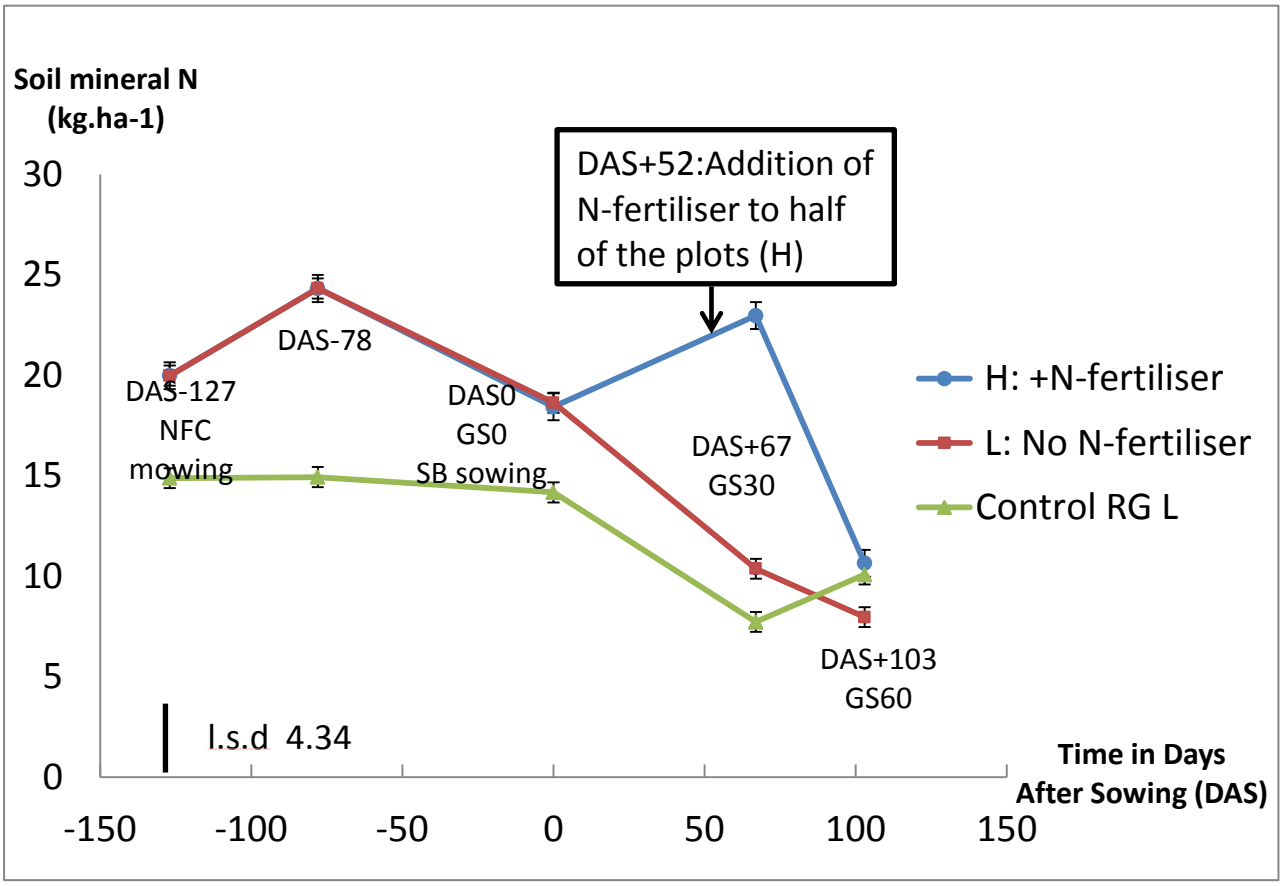

Fig6: Evolution of the average SMN (soil mineral N) content through time for the L: no N-fertiliser, H: +60kg.ha^{-1} of N-fertiliser and the control RG (Ryegrass) with no N fertiliser (L). Time was expressed in Days After Sowing (DAS) the spring barley. The vertical bars represent the standard error of the difference between means.

The average SMN division between nitrate and ammonium according to the NFC and N-treatment was shown in the graph **fig7**. For each treatment the amount of ammonium was much lower than the amount of nitrate into the soil. RG showed the lower average level of SMN. Without N-fertiliser (L) (**fig7a**), PE, WC, Mix D, Mix A, RC, Mix E, Mix C and WV, in increasing order, were significantly higher from RG. With addition of N-fertiliser (H) (**fig7b**), only Mix C and WV, in increasing order, were significantly higher from RG. WV performed a significantly higher SMN content for both L and H, followed closely by Mix C. The amount of nitrate and ammonium were both significantly higher for the H (**fig7b**) than for the L (**fig7a**) (respectively, $F_{15;446}$=13,65; p <0,001 and $F_{15;446}$=9,28; p=0,002). The soil nitrate content was found to be significantly different for the NFC treatments ($F_{15;446}$=4,62; p <0,001), with Mix C and WV being the higher for both L and H N-fertiliser, while the amount of ammonium according to the NFC treatments was not significant.

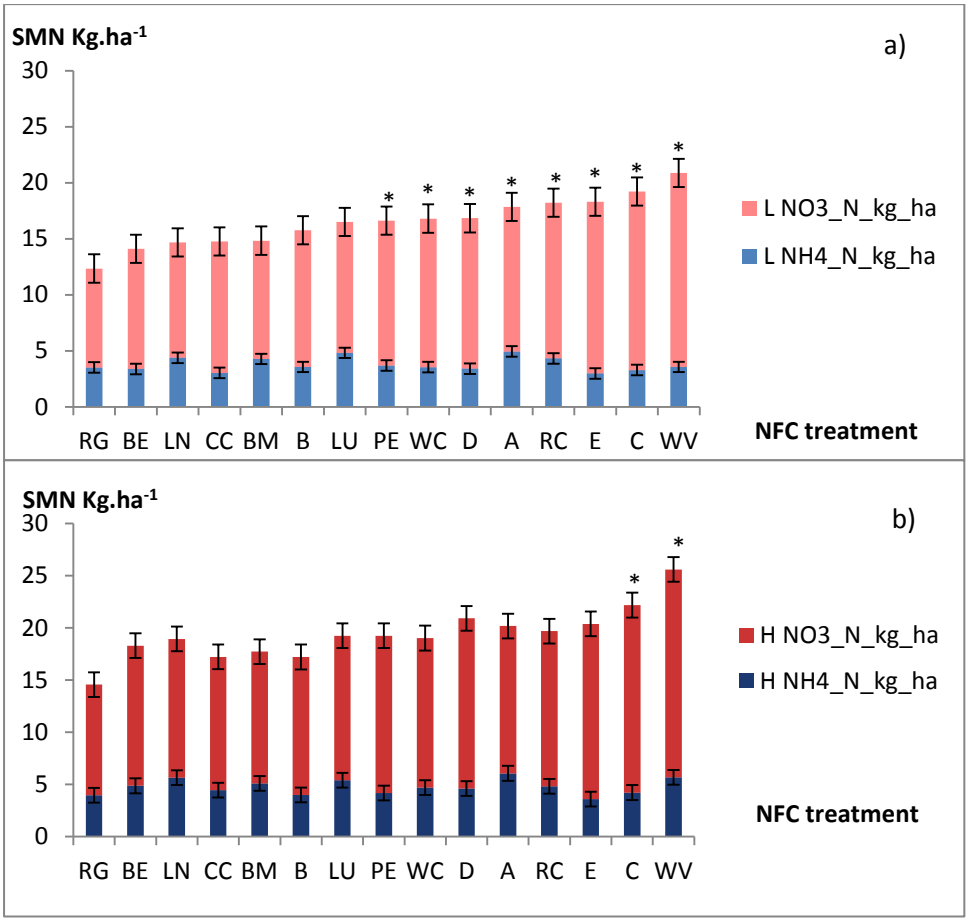

Fig7: Ammonium and nitrate repartition in the average SMN (soil mineral N) content for the NFC (nitrogen fixing crops) treatments and the control RG (Ryegrass) a) when no N-fertiliser was added (L) to the NFC treatments and b) when 60kg.ha^{-1} of N-fertiliser was added (H) to the NFC treatments. The * indicates a significant difference (P < 0.05) from RG but does not indicate the level of significance (Fisher's LSD test). The vertical bars represent the standard error of the difference between means.

At DAS-127 (no N-fertiliser treatment) (**fig8a**), LN, Mix B, Mix E, WV and Mix C soil mineral N (SMN) were significantly higher from RG (14,88 kg.ha^{-1} of SMN). At DAS-78 (no N-fertiliser treatment) (**fig8b**), they were all significantly higher from RG (14,93 kg.ha^{-1} of SMN) except LN. For most of the NFC treatments the highest SMN was recorded at DAS-78.

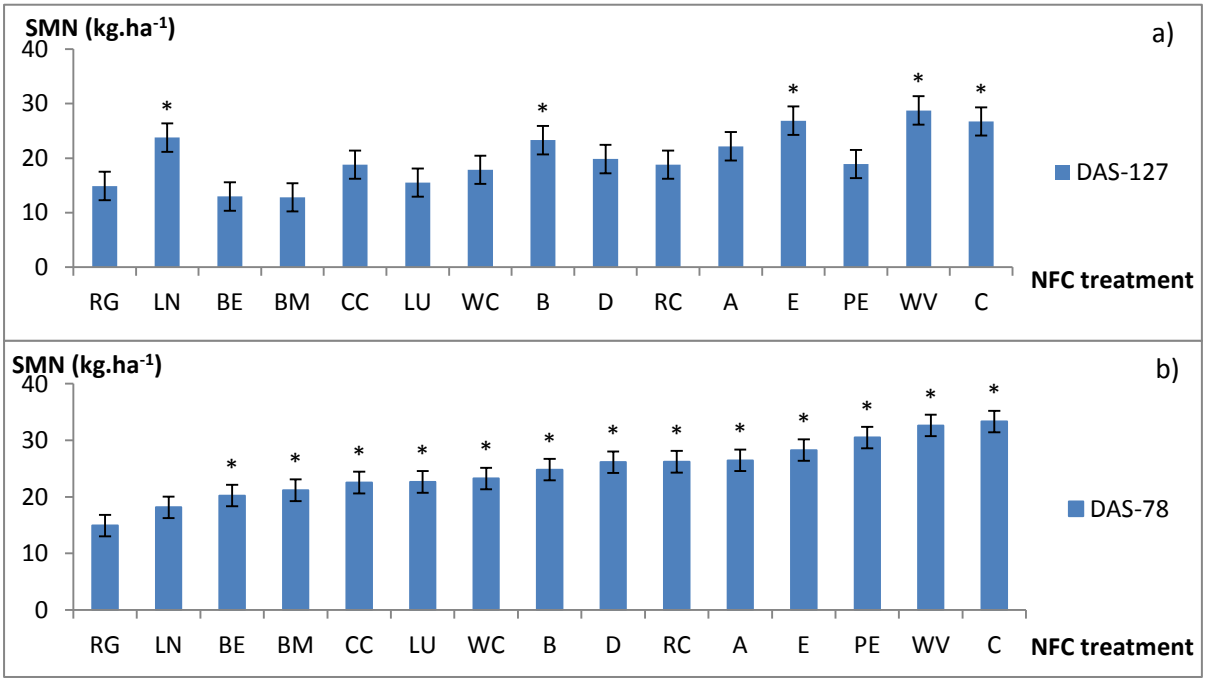

Fig8: SMN (soil mineral N) according to the NFC treatments without fertiliser (L) at a) DAS (days after sowing)-127 and b) DAS-78. The * indicates a significant difference (P < 0.05) from RG but does not indicate the level of significance (Fisher's LSD test). The vertical bars represent the standard error of the difference between means.

At DAS0 (**fig9a**), approximately the same amount of SMN was observed between the L and the H N-fertiliser for each NFC treatment. At this time in the experimentation, no plots had received any N-fertiliser treatment. The plots were split between L and H to ensure that they would have the same amount of N into the soil after receiving the exact same treatment, which was the case here. At DAS+67 L (**fig9b**), the SMN was significantly higher for LU, WC, RC and Mix A; while for DAS+67 H, LN, BE, LU, Mix D and WV had significantly higher SMN from RG. The other NFC treatments were not significantly different. CC, mix B and mix E were the NFC treatment that presented the lowest SMN content at DAS+67 for both L and H N-fertiliser treatment. For all the treatments, the SMN decreased until very low (around 6-12kg.ha^{-1}) at DAS+103 (**fig9c**). While the NFC treatments did not show a significant

difference at this stage, the N-fertiliser treatment did ($F_{1;92}$=25,13; p <0,001). At DAS+103, the SMN is significantly higher for the plots that received N-fertiliser (H).

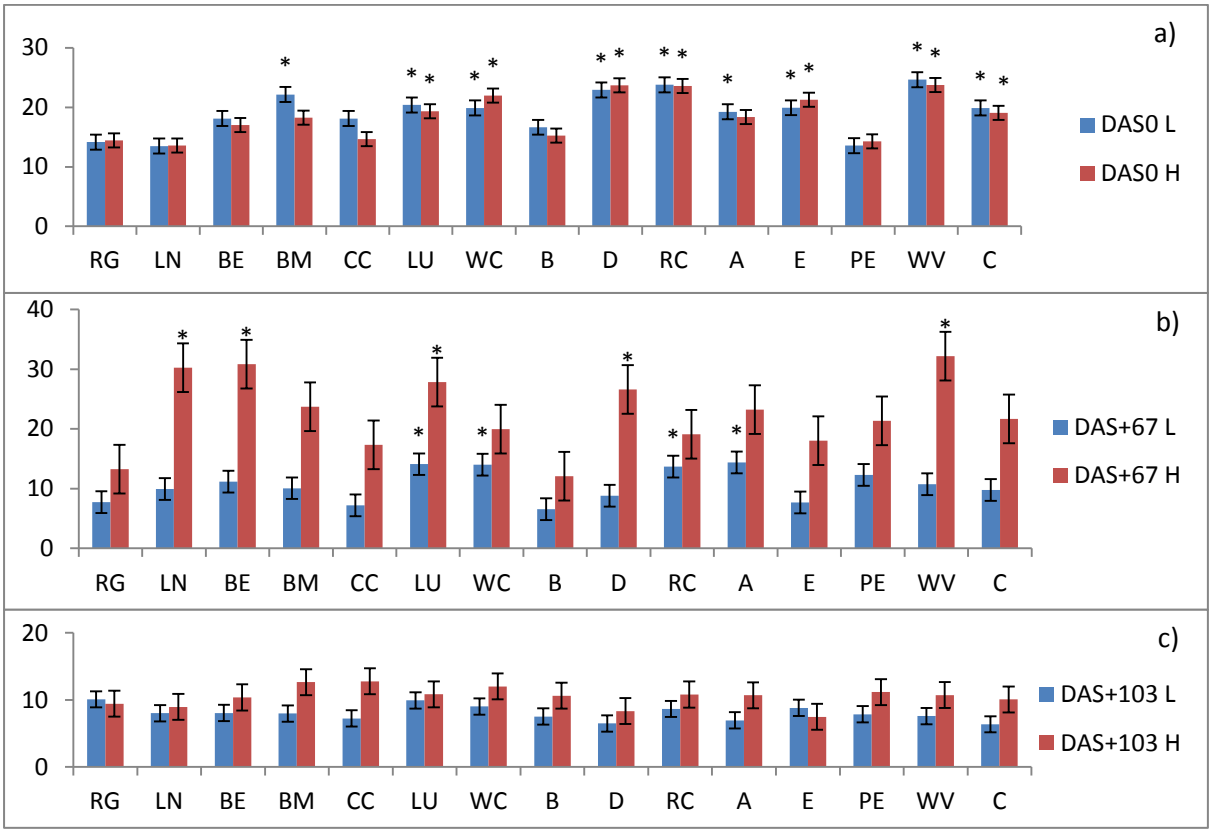

Figure 9: SMN (soil mineral N) according to the NFC treatments without N-fertiliser (L) and with N-fertiliser (H) at a) DAS0, b) DAS+67 (which correspond to the growth stage 30 (GS30) of the spring barley (SB)) and c) DAS+103 (which correspond to the GS60 of the SB). The * indicates a significant difference (P < 0.05) from RG but does not indicate the level of significance (Fisher's LSD test). The vertical bars represent the standard error of the difference between means. ATTENTION, in a), even though no plots had received the N-fertiliser treatment yet, sampling was divided between the plots that will receive L and H treatments, to verify that before beginning the different N-fertiliser treatments, they both had a similar amount of N.

3.3. Spring barley (SB) biomass production

Not surprisingly, the crop biomass (**fig10**) was significantly higher at GS60 (DAS+103) than at GS30 (DAS+67) for every treatment ($F_{1;188}$=942,42; p <0,001). At GS30 (**fig10a**), the crop weight once dried showed no significant difference between L and H. For weeds at GS30, approximately the same weight was observed for the dried L and H. Between GS30 and GS60, an herbicide was sprayed and as a result, at GS60, the weeds' biomass was too little (most of the time null) to be taken into consideration.

At GS30 the different NFC treatments have shown a significant difference between their

biomass ($F_{15;78}$=2,06; p =0,021), while no significant difference was found in their biomass at GS60.

At GS30 (**fig10a**), 2 weeks after the N-treatment was added, there was no significant difference between the L and H N-fertiliser treatment on the barley biomass. At GS60, the NFC treatments with H N-fertiliser had a significantly higher biomass than the L ($F_{1;92}$=11,77; p <0,001), with an average of 7670 kg.ha^{-1} for the L N-fertiliser and an average of 8988 kg.ha^{-1} for the H N-fertiliser, thus, 15% higher.
As said, at GS60, no significant difference was shown between the NFC treatments, thus, the biomass differences must be treated with care. The NFC with the higher biomass without N-fertiliser (L) at GS60 (**fig10b**) were, by increasing order, RC (8374 kg.ha^{-1}), mix B (8435 kg.ha^{-1}), WC (9681 kg.ha^{-1}) and WV (9899 kg.ha^{-1}). The NFC with the higher biomass with N-fertiliser (H) were, by increasing order, LU (9575 kg.ha^{-1}), mix C (10395 kg.ha^{-1}), WV (11301 kg.ha^{-1}) and mix D (11487 kg.ha^{-1}). RG, as a control, performed 7123kg.ha^{-1} for the L and 7860kg.ha^{-1} for the H. Its level of biomass production was in the lower performing half part. WC was the only NFC treatment that seemed to perform a better yield without N-fertiliser than with it.

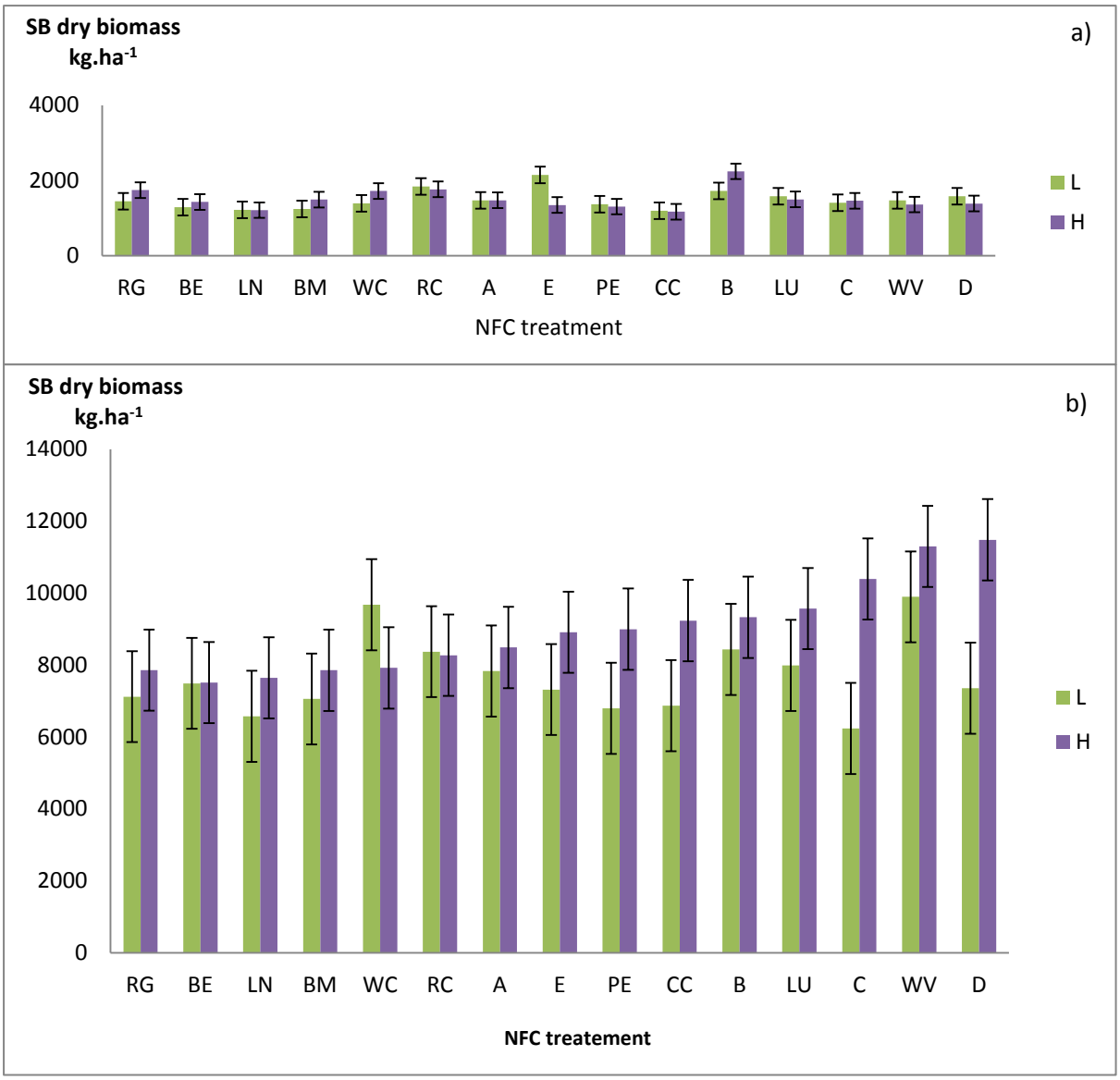

Fig10: SB (spring barley) dry biomass in kg.ha^{-1} per NFC and N-fertiliser treatments; L: no N-fertiliser and H: addition of 60 kg.ha^{-1} of N-fertiliser at a) growth stage (GS) 30 and b) GS60. RG: ryegrass; BE: beans; LN: lupin; BM: black medic; WC: white clover; RC: red clover; A: Mix A (red clover, lucerne and black medic); E: Mix E (white clover, black medic and vetch); PE: peas; CC: crimson clover; B: Mix B (red clover, white clover and crimson clover); LU: lucerne; C: Mix C (white clover, peas and vetch); WV: vetch; D: Mix D (white clover, beans and vetch) . The vertical bars represent the standard error of the difference between means.

From Spearman test, no correlation was found between the SB biomass at GS60 (DAS+103) (**fig10b**) and the SMN content when the SB was sown (DAS0) (r=0.149 ; p=0.148) (**fig9a**) while a correlation was found between barley biomass at GS60 and the SMN content at DAS-78 (r=0,259; p=0,010) (**fig8b**) and DAS+67 (r=0,234; p=0,021) (**fig9b**).

3.4. Biomass Nitrogen Use (BNU)

The values of the BNU for the treatments with N-fertiliser (H) were between 97 for WC up to 137 for Mix B of kg of biomass created per kg of N ($kg.kg^{-1}$), while the BNU without N-fertiliser (L) were spread between 313-507 $kg.kg^{-1}$ (**fig11**). Without N-fertiliser (L), the mix B performed the highest BNU (507), followed by RG (502) which is the control, PE (501), LN (488) and WC (486); while Mix C, BM, mix D, RC and mix E performed the lowest BNU (respectively 313, 319, 321, 352, and 367). The BNU for H N-fertiliser treatment seemed similar between the NFC treatments, while the BNU for the L N-fertiliser treatment showed a wider range of profile. The BNU without N-fertiliser (L) were, by 2-5 folds, higher than with addition of N-fertiliser.

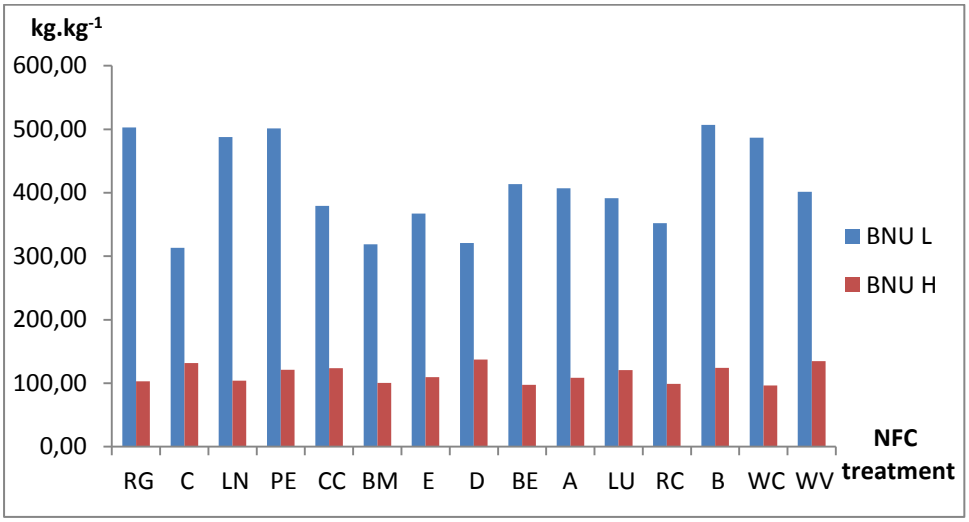

Fig11: Biomass Nitrogen Use (BNU, expressed in kg of spring barley biomass at GS60 created per kg of N in the soil at GS0) according to the different NFC treatment with (H) and without (L) addition of 60kg.ha^{-1} of N-fertiliser. RG: ryegrass; C: Mix C (white clover, peas and vetch); LN: lupin; PE: peas; CC: crimson clover; BM: black medic; E: Mix E (white clover, black medic and vetch); D: Mix D (white clover, beans and vetch); BE: beans; A: Mix A (red clover, lucerne and black medic); LU: lucerne; RC: red clover; B: Mix B (red clover, white clover and crimson clover); WC: white clover; WV: vetch.

4. Discussion

4.1. Soil fauna feeding activity

The assessment of the average fauna feeding activity was shown to be higher closer to the surface (in the top cm) and to decrease with the depth. Negar Moghimian et al., 2013 showed that the fauna biomass was higher in the 0-10cm top layer of the soil than in the following 10-20cm, which could explain a higher feeding activity in the upper part of the soil. Even though the scale is different in our experiment (0-8 cm deep), it is possible that the repartition of the fauna biomass followed the same pattern (i.e. highest biomass in the top cm which decreases with the depth) in the 8 first cm of the soil. The fauna division into the

soil could be explained by, being an interface between the soil, the air, plants/roots organic matter and water, the first centimetres of the soil are a hot spot for life colonisation.

For each of the NFC treatment, the soil fauna feeding activity was higher from the control. It might be explained by the constant cover of the soil while the control was a SB field with stubble/fallow over winter. Besides, cover crop provides environmental conditions (habitat, moisture, organic matter availability, temperature) favourable for the proliferation, activity and diversity of the soil micro- and macro- fauna (Snapp et al, 2005; Fageria et al, 2005). These results were consistent with previous studies (Debosz et al., 1999; Mendes et al., 1999; Hartwig and Ammon, 2002) where it was observed that on both short and long term, soil micro- and macro- fauna biomass and biological activity increased significantly compared with conventional systems.

Thus, NFC used as cover crops had a positive impact on the soil fauna. Some NFC treatment performed better than other, with RC (red clover) and Mix A (red clover, lucerne and black medic) showing the higher soil feeding activity. It is difficult to explain why some previous NFC treatments increased the soil fauna feeding activity while the SB is growing. It might be due to many factors, such as a difference in root network density of the NFC, a different mineralisation rate speed, a different SMN content or the partial death of the NFC due to the winter and/or to being cut[1]. It could be interesting to see if these differences would widen or disappear with repetitions of this experiment.

A correlation between the fauna feeding activity and the SMN content was expected since the soil life is triggered by soil's fertility and interferes in the SMN cycling (Snapp et al, 2005; Fageria et al, 2005). But no correlation was found when comparing the soil fauna feeding activity per NFC treatment to their respective SMN content at GS0, GS30 and GS60 and to their biomass at GS60 and their BNU. It is possible that the feeding activity of the soil's fauna had a negligible impact on the SMN content and SB biomass production.

To be more relevant, the assessment of the fauna feeding activity should have been done on every previous NFC treatment and on both L and H N-fertiliser treatments. It would have been interesting to see how the input of N-fertiliser affects the soil fauna activity. This experiment was asked by the campus of SRUC Edinburgh and the student was not in charge of the decisional part.

[1] NFC treatments were mowed at DAS-127.

4.2. Evolution of the soil mineral N (SMN) content

Nitrate (NO_3-N) and Ammonium (NH_4-N) are the two forms of N available for the plant. Their soil concentration depends on different factor such as temperature, moisture and biological activity. Ammonium-nitrogen concentration in soil is typically between 2-10 ppm (Horneck et al. 2011). The Nitrate-nitrogen may vary widely. Typical values for non-cultivated area are between 5-10ppm. Cultivated area, and therefore most of the time fertilised area, may have a rate up to 30 ppm and more. Here, the average amount of NH_4-N registered ranged between approximately 3-6ppm and the average amount of NO_3-N between approximately 9-20ppm. These NH_4-N and NO_3-N amounts were normal ranges given the agricultural parameters (agricultural land, application of fertiliser and use of GM).

At stage “DAS-127”, the NFC were mowed and removed from the field. Consequently, at DAS-78, the SMN content increased, which was likely due to the death of some plants and their roots decay releasing their N into the soil. The RG did not have this increase in SMN content , which could mean that it was less sensible to being cut. This is likely since legumes such as peas and beans would be very sensitive to the mower due to their high morphology while clovers for instance with their lower morphology could survive more easily and this might be truer for RG. Also, RG is a cereal, not a legume so it might have a lower N content, and thus, less N to release. The winter weather (frost, lack of light, cold temperature) might as well have participated in an important part of the NFC’s death. While some species such as beans and white clover can easily overwinter, some others such as peas and crimson clover are known to be frost sentitive.
Thus, due to the anticipated death of the cover crops, a part (which importance is not known) of their N content was released too early for the subsequent SB to catch it and was most likely lost.
It is likely that between DAS-78 and DAS0 the SMN content, for L and H N-fertiliser, had decreased lower than the value shown on the graph (**fig6**) and risen up again at DAS-8 when the GM were ploughed in. Similarly, it is likely that the SMN content for the H between DAS0 and DAS+67 had decreased exactly in the same way than L, until DAS+52 when the N-fertiliser was added when it suddently rose, higher than the value obtained at DAS+67, and started to decrease again to reach the value obtained at DAS+67. These suppositions could be verified by doing more SMN analysis along the experiment, especially at DAS-8, DAS+51 and DAS+53, which respectively correspond to “NFC being plough in”, “one day before adding N-fertiliser to the H”and “one day after adding N-fertiliser to the H”. This could give a more accurate profile of the N fluctuation in the soil according to the different treatments.

The SMN rates, on average, were the highest for WV and mix C for both L and H N-fertiliser treatments (**fig7**). When checking their crop biomass at GS60, WV was found to have one of the highest yield for both L and H while mix C had one of the highest biomass as well for H but one of the lowest for the L. It is difficult to conclud on the link between the SMN content and the crop biomass since no correlation was found between the SB biomass at GS60 (DAS+103) and the SMN content when the barley was sown (DAS0), while a correlation was found between SB biomass at GS60 and the SMN content at DAS-78 and DAS+67. The correlation between the SB biomass at GS60 and the SMN content at DAS+67 is most likely due to the addition of N-fertiliser to half of the plots rather than to the different NFC treatments, which does not mean that they have no impact but that it might be negligible beside N-fertiliser treatments.

At DAS+67 it was observed that without N-fertiliser (L) (**fig9b**) the NFC treatments that had the highest SMN content were all clovers (Lu, WC, RC and mix A) while for the H N-fertiliser (**fig7d)**, the 3 categories, clovers, grain legumes and mix of both, were present in the top 5 highest SMN content (Ln, Be, Lu, mix D and Wv). This could mean that clovers were more efficient at catching up/fixing N from the soil/air than the other NFC treatments and that the subsequent addition of N-fertiliser invisibilise this advantage by flooding this differences.
At DAS+103, all NFC treatments, for both L and H ended up with similar (even though significantly different), low level of N in the soil (**fig6, fig9c**). These could mean that crops have removed all the N they could from the soil. It might be possible that under a certain concentration of N in the soil, the plant could not remove it effectively anymore. This results are consistent with the fact that plants are generally more limited by the N availability in soil than by their uptake capacity (Thorup-Kristensen et al, 2003). If plants had taken the same amount of N, the SMN content would still have the same proportionnal difference at DAS+103 that at DAS+67. These results are still to be verified by assessing the N content of the crops. The similar amount of N in soil between the treatment could be due in part to other factors such as N leaching.

4.3. Spring barley (SB) biomass production

The crop biomass production was observed to be significantly higher for the H than for the L N-fertiliser treatment at GS60 (DAS+103) (**fig10b**) while no difference was observed at GS30 (**Fig10a**). For GS30 measures, the N-fertiliser had been added only 15 days (DAS+52) before the sampling date, thus, it might have been too early to see any growth difference between the L and the H N-fertiliser treatments.
While the NFC treatments do have differences between their biomass, it does not appear to

be enough to be significant. Significant differences might appear in longer term studies. Thus, for now, the differences have to be treated with care. While WC seems to have performed a higher crop biomass with no N-fertiliser (L) (**fig10b**), this needs further investigation to assure that this result is consistent. WC was as well found to have one of the highest BNU for L N-fertiliser treatment (**fig11**).

While a significantly different biomass according to each NFC treatment was found at GS30, it was not found for GS60. It is possible that the addition of N-fertiliser might lower/smoother the differences between the NFC treatments.

The crop biomass results might be biased since spring 2017 was not good for crop establishment due to climatic conditions (dry weather just after sowing), and it might have affected the crop biomass productivity. As a consequence, the barley field was very patchy, and for the same NFC treatment, some replicates were very different which is why the analysis of variance shows no significance.

It would have been interesting to have a biomass control where SB would have been grown with no previous NFC nor N-fertiliser treatment to evaluate what was directly due to the NFCs.

The different NFC-treatments did not have a big effect on the aboveground barley biomass, which could change with time. Repetition of this experimentation could allow to confirm or to set aside a possible effect of the different NFC-treatments, but it is likely that they do not have a different effect because they are in fact biologically and in their N-fixing activity very similar.

4.4. Biomass Nitrogen Use (BNU)

The BNU was much higher for the treatments without (L) than with (H) N-fertiliser. Adding N-fertiliser considerably lower the BNU efficiency. The 60 $kg.ha^{-1}$ of N-fertiliser added to the H treatment increased its biomass production by only 15% but decreased the BNU by 2-5 folds. To show a BNU as high as without N-fertiliser, the biomass of the SB should have increased proportionally to the quantity of N applied.

The addition of N-fertiliser, in terms of BNU, seemed to be of poor efficiency. It is possible that the SB reached a plateau where its biomass could not have increased more and/or that the N-fertiliser was in part lost, maybe through leaching process. When measuring the SMN content at DAS+67, 15 days after the addition of the 60$kg.ha^{-1}$ of N-fertiliser to the H, only 12-30$kg.ha^{-1}$ of SMN was found. While we do not know the proportion of N that the plant would take off, it is possible that most of it had been lost through leaching process and/or that the SMN measures were not representative of the quantity of N in the soil. The N analysis of the crops and the grain yield would give more indications on the NUE.

Moreover, in view of the results, it seemed that the addition of N-fertiliser smothered/lowered the BNU differences in-between the NFC treatments (**fig 11**), which could be due to the fact that the amount of N that each cover crop was able to fix was flooded in the amount of N applied as fertiliser.

When no N-fertiliser was applied, the different categories of NFC; clovers, grain legumes and mixtures (as a reminder, see **fig1 and appendix3 and 4**), does not seem to influence their BNU, since the 3 categories were found in the 5 highest as well as in the 5 lowest BNU.

One of the aims of this study was to compare RG, which is a commonly used cover crop in Scotland, with NFC in an attempt to find a better performing GM in term of yield and NUE, to combine the N fixing activity to add N into the soil, and the effective removal of N from the soil. While RG had one of the lowest crop biomass for both L and H N-fertiliser treatments, it performed the 2nd highest BNU. Not surprisingly, RG seemed to be one of the best performing in term of efficiency to remove the N from the soil but some NFC had similar performances (Mix B, LN, PE and WC).

Another observation is that adding N-fertiliser may improve crop biomass but lower hugely the BNU, from 2 to 5 folds lower from the treatments with no N-fertiliser. This method of calculation might not be very accurate because it does not take into account the N-fertiliser that leaches into the ground, or is lost through other processes, and thus that is not affectively available for the plant. This parameter might explain the huge difference between the BNU of the L and the H N-fertiliser treatments. As said previously, the method of measuring and calculating the SMN might also be to blame.

4.5. Experiments that could not be completed

Due to conditions outside of the student's control the SB N content analysis at GS30 and GS60 and the decomposition rate with the TBI could not be completed on time. See **appendix 9** for a description of their methodology and expected results.
The TBI method should have given an estimation of the soil organic matter decomposition rate. The aim of this experiment was to assess if the decomposition rate could be influenced by the NFC treatments and the level of N-fertiliser.
The higher is the C:N ratio, the slower is the rate of decomposition (Yadvinder-Singh et al, 1992) and legumes have a much narrower C:N ratio than cereals (Lauringson et al, 2013). Thus, the results expected would have been to find a similar decomposition rate for all the NFC treatments due to their low C:N ratio and a lower decomposition rate for the RG. A higher decomposition rate was expected for the plots which received the H N-fertiliser

treatment since high soil mineral N content favours a quicker decomposition (Yadvinder-Singh et al, 1992).

4.6. On NUE and its components

They are different ways to calculate the NUE and the results may vary widely according to which method is used. Bingham et al (2011) have tried 3 different methods to estimate the Nitrogen supply (Ns), which all show different results. In this study, the NUE was estimated as the grain yield (Gy) according to the Ns (NUE=Gy/Ns). The 1st method, after Huggins and Pan, (1993) gave Ns as the N off-take without fertiliser plus the residual SMN post harvest plus the N applied as fertiliser (1: Ns=Noff zero fert+ rSMN post-harvest + Nf), while the 2nd method gave the Ns as the N off-take without fertiliser plus the N applied as fertiliser without taking into account the residual SMN post harvest (2: Ns= Noff zero fert + Nf). The 3rd method gave the Ns as the SMN pre crop emergence plus the N applied as fertiliser (3: Ns=SMN pre-emergence+ Nf). Method 2 increased the values of NUE of 58% in comparison with the method 1, while method 3 was in-between. This study shows that the method used to estimate N supply has a big effect on the estimation of the NUE. Comparing results from different studies must be done with care, taking into account the different methods used.
In the case of our experiment, if the SB N content could have been analysed, a NUE could have been estimated. The Ns could have been calculated according to the method 1 (Ns=GS0 SMN+ N applied as fertiliser+ residual SMN post harvest) and the NUE could have been estimated as the amount of N in the plant at GS60 per the Ns (NUE= plant N GS60/Ns) which could have given a proportion of N used according to the N available. The relevance of calculating NUE according to the crop N content at GS60 rather than with the grain yield was a time matter in the framework of the student's thesis. Since it could not be carried out on time anyway, it can be replaced by the grain yield in the planned calculation.

4.7. Balance between the economic, agronomic and environmental issues

Another purpose of this experiment was to study and rectify, if needed, the N-fertiliser rate recommended (SAC Consulting, the farm management handbook 2016/2017) to grow SB after specific crops such as LN, BE, PE or RG. According to the preceding crop, the N-fertiliser rates needed are not the same to grow SB. Some crops leave more N in the soil than others. This assessment cannot be carried out before all the results are known (N content of the SB and grain yield). However, what can be said now is that a balance between the economic, agronomic and environmental issues should be done. While the NFC can provide some of the N the plant needs, agricultural systems without N-fertiliser inputs might see a yield loss in comparison with systems where N-fertiliser is applied. The yield

loss does not necessarily mean an economic loss. In some systems (i.e. organic), the yield loss can be balanced by saving the cost of the inputs, by direct selling, added value products, high quality products and many other ways. Overall, NFCs, as being less damaging for the environment than the use of N-fertiliser should be encouraged, when possible.

5. Conclusion

The NFC treatments increased the soil mineral N content in comparison with ryegrass and triggered the soil fauna activity in comparison with field with fallow over winter. They had no significant effect on the SB biomass yield at anthesis in comparison with RG and in-between them. The soil fauna activity did show no visible effect on the BNU and crop biomass production. The addition of N-fertiliser has shown to improve the SMN content and the biomass of the SB, and supposedly will improve the grain yield. The BNU of SB varied accordingly to the previous NFC and N-fertiliser treatments. The application of N-fertiliser drastically decreased (from 2-5 folds lower) the BNU while it improved the crop biomass production by 15%. This shows that the N brought by the fertiliser was very inefficiently caught up by the crops. While the BNU of the NFC treatment with addition of N-fertiliser seemed all similar, without N fertiliser they have shown interesting differences; the control (RG) had one of the highest BNU, alongside with LN, PE, Mix B and WC, which suggests that some legumes could remove as effectively N from the soil than grass while fixing N from the atmosphere. The relevance of the BNU calculation is questionable since it is less than certain that it represents reliably the NUE.
Using legume-based green manure is certainly lowering the environmental cost of using N-fertiliser. By mixing these two sources of N, a balance can be made between lowering the environmental impact of fertiliser and maintaining a competitive yield.

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Appendices:

Appendix 1: Historic Saphock Farm

2013: The field was certified organic, growing grass.
2014: The field was passed to conventional for SRUC's needs. SB was cultivated with 125kg.ha^{-1} of N-fertiliser.
2015: Winter barley was cultivated with 175kg.ha^{-1} of N-fertiliser.
2016: NFC were grown for this current study.
2017: SB was cultivated for this current study.

Appendix 2

STRAIGHTS	Seed rate g/m^2
Lucerne LU	2
Black medic BM	1
Beans BE	20
Vetch WV	6
Lupin LN	16
Peas PE	15
Red clover RC	1
White clover WC	0.7
Crimson clover CC	1

Table 2: Seed rates of the NFC straight treatments

Appendix 3

MIXTURES seed rate g/m^2	A	B	C	D	E
Red clover RC	0.33	0.33			
White clover WC		0.23	0.23	0.23	0.23
Crimson clover CC		0.33			
Lucerne LU	0.66				
Black medic BM	0.33				0.33
Beans BE				6.6	
Peas PE			4.95		
Vetch WV			2	2	2

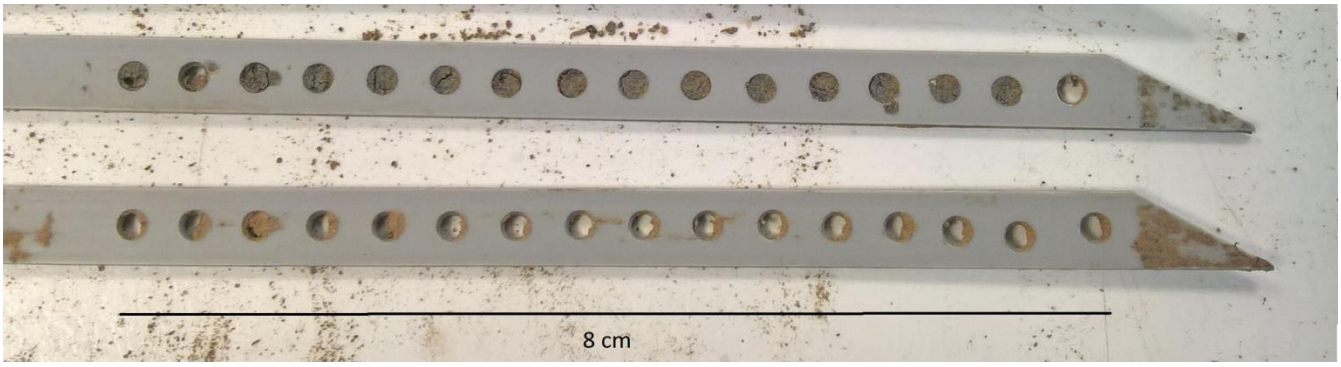
Table 3: Seed rates of the NFC mixture treatments

Appendix 4: Student's role

The role of the intern student was to prepare the sampling sessions, go to the experimental field to sample and collect data, process the data collected on the computer system, carry out the lab work that resulted from the field sampling and to record field notes and details of all operations (including type, and rate of any inputs).

Data analysis of the SMN content was not carried in SRUC Aberdeen but in SRUC Edinburgh campus, because they had the device Skalar, San++ continuous flow colorimetric analyser needed for the analysis. The intern student carried out the field and lab work alongside with SRUC Technical Officer, Derek Simpson and other interns.

Appendix 5



Picture 1: Two bait-lamina sticks just being dug up from the field; the one on the top is mostly filled with bait lamina, the bottom one is empty.

Appendix 6

Picture 2: Spring barley 23/06/2017, 10 days after GS30. Saphock Farm, Aberdeenshire, Scotland

Appendix 7

Picture 3: Spring barley 19/07/2017, GS60 Anthesis. Saphock Farm, Aberdeenshire, Scotland

Appendix 8

Barley growth stages and benchmarks

Growth stages

Growth Stage	Description of stage
	Seedling growth
GS10	First leaf through coleoptile
GS11	First leaf unfolded
GS13	3 leaves unfolded
GS15	5 leaves unfolded
GS19	9 or more leaves unfolded
	Tillering
GS20	Main shoot only
GS21	Main shoot and 1 tiller
GS23	Main shoot and 3 tillers
GS25	Main shoot and 5 tillers
GS29	Main shoot and 9 or more tillers
	Stem elongation
GS30	Ear at 1cm (pseudostem erect)
GS31	First node detectable
GS33	3rd node detectable
GS35	5th node detectable
GS37	Flag leaf just visible
GS39	Flag leaf blade all visible
	Booting
GS41	Flag leaf sheath extending
GS43	Flag leaf sheath just visibly swollen
GS45	Flag leaf sheath swollen
GS49	First awns visible

Growth Stage	Description of stage
	Ear emergence
GS51	First spikelet of ear just visible
GS55	Half of ear emerged
GS59	Ear completely emerged
	Flowering
GS61*	Start of flowering
GS65	Flowering half-way
GS69	Flowering complete
	Milk development
GS71	Grain watery ripe
GS73	Early milk
GS75	Medium milk
GS77	Late milk
	Dough development
GS83	Early dough
GS85	Soft dough
GS87	Hard dough
	Ripening
GS91	Grain hard (difficult to divide)
GS92	Grain hard (not dented by nail)

*in some spring crops GS61 may precede GS59

Barley growth stages

The Decimal Code system for measuring barley growth used throughout this guide is based on work published in Tottman, DR, Makepeace, RJ and Broad, H (1979) An explanation of the decimal code for the growth stages of cereals, with illustrations. Annals of Applied Biology **93**, 221–234.

Table 4: Barley Growth Guide, AHDB, Cereals and oilseeds: Barley Growth Stage and Benchmarks

Appendix 9: Experiments not completed

- Material & Method:

- Decomposition rate:

The decomposition rate in each plot should have been estimated by the Tea Bag Index (TBI) method as described by Keuskamp et al (2013), to compare the decomposition rate following the different NFC treatments. The specific protocol to our study can be seen in the **appendix 10**. Standardised Tea bags (Lipton brand) were buried per pair of one rooibos and one green tea bag, 5 weeks after barley sowing, for 90 days. Five replicates were done in every of the 96 plots, which make a total of 480 pairs of teabags used in this experiment. Each tea bag has been weighed before being buried.
The teabags were buried in mid-May 2017 (12th, 15th and 16th of May). Target date for bag removal was mid august.
The teabags weight before burial was slightly different for the Red tea and the Green tea. The average weight for the 480 green teabags was 2,11g and the average weight for the 480 red teabags was 2,26g. The experiment began with a difference of 0,15g between the 2 sorts of tea. This weight difference should have been taken into consideration when comparing the teabags weight after digging up.
Unfortunately, due to time limitation this experimentation could not be completed.
After being dug up, the teabags should have been oven-dried and weighed again without the bag mesh, string and label. The weights before and after burial should have been compare and the weight loss would have represented the decomposition rate for each NFC treatment.
In my opinion a control should have been done with a field of conventional SB with bare fallow over winter such as the one used for the bait-lamina sticks.

- Spring barley N content:

At GS30 and GS60 aboveground SB samples were taken from a 0.5x0.5m random quadrat. The samples were separated into crop and weeds. Samples were dried at 80°C and a subsample was milled and stored for N content analysis. Due to a technical fault with the mass spectrometer, this could not be done on time.

- Results expected:

- Decomposition rate:

What was expected was a loss of biomass of the tea bags, which would have represented the decomposition rates of the two sort of tea. Their decomposition rate could have varied according to the NFC treatments. However, a profile like the bait-

lamina stick would be more likely (i.e similar results between all the NFC treatments), since the soil life activity is strongly correlated with the organic matter decomposition. Thus, the observed result might have been a similar loss of biomass for all the different NFC treatments, and a similar decomposition rate. We expected to observe a more important decomposition rate when additional N was applied as N-fertiliser, since the decomposition rate increase with the amount of N in the soil (Thorup-Kristensen et al, 2003).

- Spring barley N content:

It can be assumed that the SB N content would have been higher for the half of the plots that received the H N-fertiliser treatment. Predicting how the crop N content would have evolved according to the different NFC treatments is trickier, but it could be assumed that the NFC that released the more N into the soil when ploughed in would have the higher subsequent crop N content.

Appendix 10: Tea Bag Index protocol

TBI protocol (Text in grey, from step 6 to 10, could not be completed):

1. Use one bag of Lipton green tea (EAN: 87 22700 05552 5) and one Lipton rooibos tea (EAN: 87 22700 18843 8) per replicate.
a. To obtain better estimates of TBI, 5 replicates were buried per plot, with a total of 96 plots.
2. a. Determine the airdry weight of 5 empty bags, separated in bag mesh, string and label
b. Determine the weightloss of tea of those 5 bags by placing them 24h in an oven at 105 C.
c. Measure the initial weight of all the tea bags and subtract the weight of an empty bag to determine the initial weight of the tea.
3. To mark the tea bags, stick two labels on each side of the tea bag's label using a permanent blackmarker.
4. Bury the tea bags in 8-cm deep, separate holes while keeping the labels visible above the soil and mark the burial site with a stick.
5. Note the date of burial, geographical position, ecotype and experimental conditions of the site.

6. Recover the tea bags after c. 90 days
7. Remove adhered soil particles and dry in a stove for 48 h at 70°C (not warmer!).
8. Remove what is left of the label but leave the string, weigh the bags and subtract the weight of an empty bag without the label to determine the weight after incubation.
a. To get a more precise estimation, open the bag and weigh its content; combust the content at 550°C and subtract what is left from the content weight.
9. Calculate stabilisation factor S and decomposition rate k using eqn 1b. $W(t)=ae^{(-kt)}+(1-a)$ (Keuskamp et al, 2013).
10. More (facultative) instructions and tips on how to incorporate the TBI in scientific experiments can be found on our website:
http://www.decolab.org/tbi

Appendix 11: Reflection section

This master's thesis was challenging to me, especially because things did not turned out as I expected, even though I imagine it to be similar for every master's thesis. Many results were late or even missing. Many mistakes of sampling, organisation, calculation (in short every kind of mistake) were made and needed to be corrected. I had to adapt to changing conditions and to try to do my best to reframe the study's aim. Even though it was not a very comfortable situation, I think it helped me to rely more on myself, to take more responsibilities and to improve my self-confidence.

I particularly found difficult to organise and manage my time on my own. Estimating how much time is needed to do something that I never did before is quite tricky, i.e. analysing a consequent amount of results with Genstat software that I had never used.

I figured out that a way too important part of my struggles during the process of writing my thesis was due to a lack of knowledge in computer informatics and software such as Excel, Word, Genstat or Reference manager. I lost hours sometimes to solve a purely technical problem. I was thus obligated to learn a lot about it for the need of my thesis.

I think this project was a bit ambitious to cover on only five months internship; they were too many experiments in the same time, many results from the past year that were not analysed yet, the goals were not very clearly defined and many assessments should have been repeated to ensure they were significant or could have been improved in adding a control for instance. Maybe it would have been more relevant to do this project from the beginning to the end as a PhD thesis.

This experience confirmed what I already knew; I do not want to work in research in the future. Knowing that, the choice of this internship might seem surprising, but, one year ago, while looking for a master thesis, I had many other ideas. After all this ideas had failed, I decided to go for a research thesis on a topic that I was really interested in: the green manures.

More precisely, the interrogation that made me want to join this project was to evaluate under which circumstances it would be possible to fertilise the soil with green manure as the only source of N and it became clear that the major constraint is the reduction of the subsequent crop yield and consequently the economic aspect. While this is subject to controversy, it is I believe the major constraint against the widespread of GM amongst farmers.

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