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# Evaluating the use of Perennial Mobile Green Manures for crop nitrogen supply: a pot experiment

MSc Sustainable Food and Natural Resources

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## Abstract

*Nitrogen (N) is a common limiting nutrient for crop growth and adequate supply is essential to ensure food security. Due to supply and demand synchrony issues and high N mobility in the soil, applications of mineral fertilisers and green manures commonly incur high N losses to volatilisation, leaching and greenhouse gas emissions, with more than 50% of N applied lost to the environment where it causes ecosystem damage. Perennial Mobile Green Manures (PMGMs) are perennial nitrogen-fixing species that can be harvested, potentially processed/stored, and applied to the soil. Early studies show that they can generate equal or more crop biomass production and N uptake than a conventional green manure, whilst incurring lower N loss rates and lower production emission rates than mineral fertilisers. This study examined N provision of Alder (*Alnus glutinosa*), Scotch broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*) compared to red clover (*Trifolium pratense*), ammonium nitrate (AN) fertiliser and a control with no additions in a 20 week outside pot experiment. Treatments were added at the rate of 200 kg ha<sup>-1</sup> N equivalent and a kale crop was grown. Crop germination, above and below ground biomass growth, appearance and root morphology were recorded. A decomposition experiment using litter bags was also carried out for the PMGMs. All kale plants treated with PMGMs had significantly more biomass than the control ( $P < 0.01$ ), signifying N release and uptake from the PMGMs. There was no significant difference between alder, broom, red clover and AN treated plants, indicating that crop N uptake from PMGMs was adequate for crop growth. Gorse had the highest C:N ratio, slowest decomposition rate and was the only PMGM treatment to have significantly less crop biomass than the AN treatment ( $P < 0.05$ ). The decomposition rates followed the C:N ratios of the PMGMs. Kale plants with PMGM and clover treatments developed longer and more extensive root structures than AN treated plants, indicating higher climate resilience in plants with organic amendments. PMGMs can grow on marginal land in multifunctional bio-service areas adjacent to arable land, reducing the amount of arable land set aside for biological N fixation, and contributing other ecosystem services such as C sequestration, biodiversity and wind and flooding protection. This, combined with the low energy input and emissions of PMGMs whilst still providing adequate N supply, means their use could be important for a more sustainable agricultural system. The dynamics of crop uptake and soil storage of organic N may be instrumental to the functioning of PMGMs and requires further research.*

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## Glossary

BNF	Biological Nitrogen Fixation
DW	Dry Weight
GHG	Greenhouse Gas
IPCC	Intergovernmental Panel on Climate Change
NUE	Nitrogen Use Efficiency
PMGM	Perennial Mobile Green Manure
SM	Secondary metabolite
SEM	Standard Error of the Mean
SRL	Specific Root Length

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# 1. Introduction

## 1.1 Nitrogen in agriculture

The modern large-scale agricultural system is a main driver of climate change, ecosystem damage and biodiversity loss worldwide due to high CO<sub>2</sub> emissions from fossil fuel use and soil degradation, increasing use of other resources such as water, pesticides and fertilizers, poor nutrient management and leaching, and land use change (Millennium Ecosystem Assessment, 2005; Rockström *et al.*, 2017; IPBES, 2019; IPCC, 2019; Gerten *et al.*, 2020; Tubiello *et al.*, 2021). Today, almost 50% of food production transgresses the ranges for a safe and inhabitable planet for humans, the so called planetary boundaries, for biosphere integrity, land-system change, freshwater use and nitrogen (N) flows (Gerten *et al.*, 2020), with N flows being at particularly high risk (Steffen *et al.*, 2015). Simultaneously, the global food system is vulnerable to the effects of climate change such as weather and climatic changes (Godfray and Garnett, 2014; Rockström *et al.*, 2017). The agricultural system struggles to sustainably feed a growing population, and it is vital to increase agricultural output without the ecological footprint following suit (Rockström *et al.*, 2017; Gerten *et al.*, 2020; Chang *et al.*, 2021).

Nitrogen (N) is the main building block of proteins, nucleic and amino acids, and therefore essential to all organisms, but it tends to be the most limiting nutrient for plant growth as it is not freely available in the atmosphere (Bloom, 2015; Soumare *et al.*, 2020; Martínez-Dalmau, Berbel and Ordóñez-Fernández, 2021). Reactive, bioavailable N is created through biological or chemical fixation, and it is often supplemented to the soil as a chemically produced fertiliser to enable the high crop production needed to sustain a growing population (Fageria and Baligar, 2005; Hirsch and Mauchline, 2015; Abdalla *et al.*, 2019; Soumare *et al.*, 2020). The production of such mineral N fertiliser requires energy input, almost exclusively from fossil fuels, and emits CO<sub>2</sub> into the atmosphere, substantially augmenting the overall climate impact of the food system (Rosa and Gabrielli, 2023). It is estimated to make up 2% of the world's energy consumption, contributing to emission levels that are incompatible with current climate goals (Houlton *et al.*, 2019; Clark *et al.*, 2020).

There are low recovery rates of the N supplied through both organic amendments and mineral fertilisers, with excess N prone to leaching into waterways generating high N concentrations, eutrophication and ecosystem damage (Howarth and Marino, 2006) or it can return to the atmosphere in greenhouse gas (GHG) emission forms such as nitrous oxide, N<sub>2</sub>O (Cameron, Di and Moir, 2013; Batty, Aneja and Schlesinger, 2017; Houlton *et al.*, 2019; Carr *et al.*, 2020). Leaching is particularly an issue in sandy soils and with horticultural crops (Cameron, Di and Moir, 2013; Carr *et al.*, 2020). Only approximately half of applied N is taken up by the crops, leaving a significant imperative to improve N management for a sustainable food system (Gerten *et al.*, 2020; Chang *et al.*, 2021; Mahmud *et al.*, 2021). This imperative becomes even more urgent when considering the current geopolitical situation.

Fossil fuel dependent nitrogen fertiliser increases vulnerability in the food system as it becomes subject to shocks in the energy and supply market, such as the most recent disruptions caused by the Covid-19 pandemic and the war in Ukraine (Rosa and Gabrielli, 2023).

## 1.2 Green Manures

Biological N fixation occurs naturally in ecosystems through microorganisms, and can happen in symbiosis with plants (Peoples, Herridge and Ladha, 1995; Bloom, 2015; Soumare *et al.*, 2020). Such plants, most notably legumes of the Fabaceae family such as peas, beans, clovers, vetch and alfalfa, are used in agriculture to fix N and make it available for subsequent crops, and are referred to as green manures (Cherr, Scholberg and McSorley, 2006; Blanco-Canqui *et al.*, 2015; Carr *et al.*, 2020; Soumare *et al.*, 2020). Relying on green manures to supply crops with N avoids the energy use and GHG emissions of mineral fertiliser production and can contribute to increased soil organic carbon levels and soil fertility (Lehtinen *et al.*, 2014; Garcia-Franco *et al.*, 2015; Carr *et al.*, 2020). However, the green manures are often grown on agricultural land instead of cash crops, lowering the overall amount of land available for food production (Cuttle, Shepherd and Goodlass, 2003; Smith *et al.*, 2018). Additionally, the green manures are tilled into the soil where they break down and release the fixed N, meaning that there is little control over when the N is released and it is difficult to achieve synchrony between N supply and crop demand (Crews and Peoples, 2005; Chen *et al.*, 2014; Carr *et al.*, 2020). In fact, green manure systems have been shown to have similar rates of leaching and low N recovery rates as mineral fertiliser systems (Stopes *et al.*, 2002; Bergström and Kirchmann, 2004; Campiglia *et al.*, 2011).

## 1.3 Perennial Mobile Green Manures

To achieve better N supply and demand synchrony, there have been suggestions of mobile green manures, where green manures are harvested, processed and applied to crops when needed (Crews and Peoples, 2005; Sorensen and Thorup-Kristensen, 2011; Sorensen and Grevsen, 2016; Gatsios *et al.*, 2021). A very recent further exploration of this idea is the use of perennial mobile green manures (PMGMs) (Ward, 2020), consisting of woody perennial nitrogen fixing shrubs or trees, such as gorse and alder. These species not only fix N and can be added to crops, but they also grow in marginal or substandard land and are often considered invasive, meaning that agricultural land does not need to be set aside for N fixation (Ward, 2020; Ward, Chadwick and Hill, 2023).

The term PMGM has been established by others (Ward, 2020; Ward, Chadwick and Hill, 2023). In less academic contexts, the term perennial green manures is used to refer to the same species and practices (Yeomans, 2023; Ward, 2024). In the academic field however, conventional green manures such as alfalfa, clover and ryegrass can be called perennial (see e.g. Sorensen and Grevsen, 2016; Lyngge *et al.*, 2023), so to avoid confusion, the term PMGM is used in this research.

Research into PMGMs is in its infancy, with only a couple of studies carried out to date. These studies show promise in that the N supply of PMGMs can match that of



conventional green manures, but more research is needed to determine which species of PMGMs are most appropriate along with application rates, timing and methods.

#### 1.4 Research Aims and Objectives

This study has examined alder (*Alnus glutinosa*), Scotch broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*) as PMGMs native to Scotland, where the experiment took place. The aim of the experiment and main research question was

- ‘What effect do the PMGMs have on biomass growth of “Red Russian” Kale (*Brassica napus* var *Pabularia*) in sandy soil in a pot experiment?’

Three additional research questions were considered:

- ‘What effect do the PMGMs have on crop and weed germination?’;
- ‘What effect do the PMGMs have on crop root structures?’; and
- ‘What are the decomposition rates of the PMGMs?’

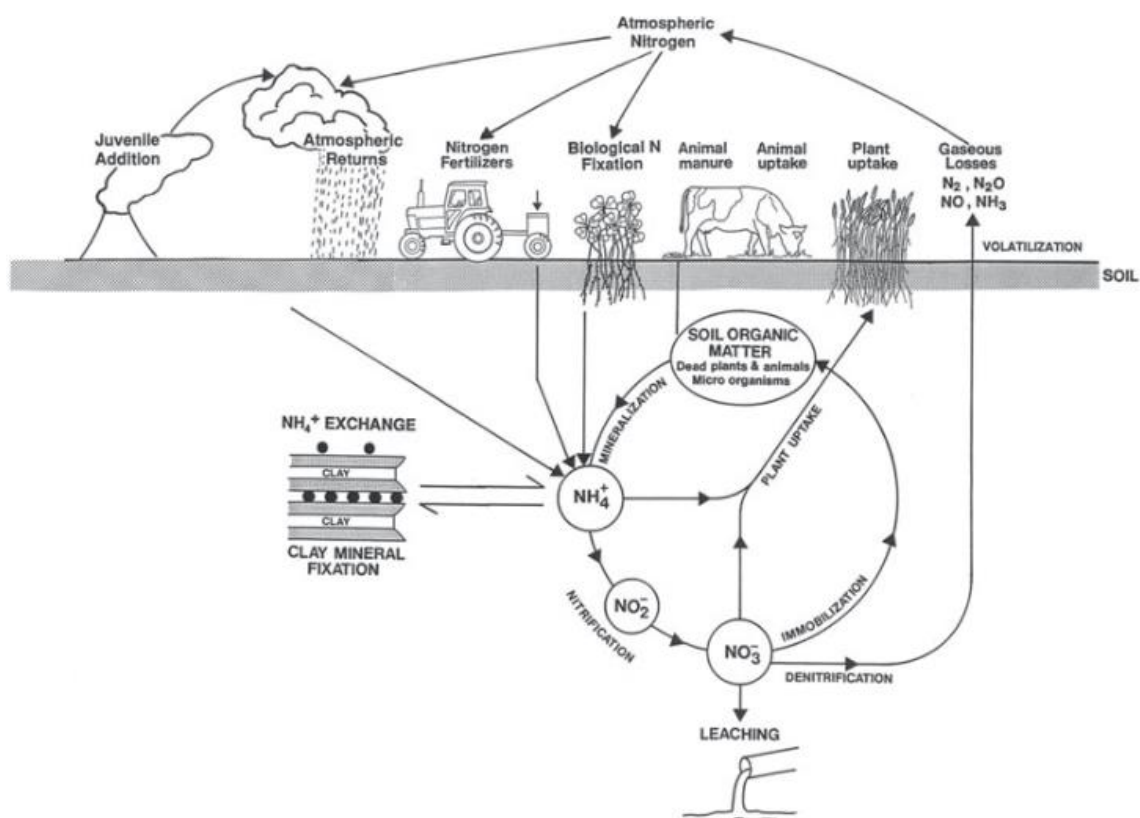
The horticultural crop was chosen as there had not yet been any studies of PMGM use in horticulture at the start of this project (Ward, Chadwick and Hill, 2023). A pot experiment was carried out to ascertain crop growth response to a controlled amount of N addition in the form of PMGMs, as well as red clover and Ammonium Nitrate mineral fertiliser for comparison. Destructive harvesting of the crop enabled data collection of above and below ground biomass growth and morphological data. Recent studies have shown that Scotch broom and gorse have an allelopathic effect and suppress weed germination (Pardo-Muras *et al.*, 2020), motivating the further research question on germination.

The overall aim of this research is to contribute to the growing field of PMGMs and examining their ecological and environmental viability as alternative sources of N fertiliser. Importantly, this study set out to assess the impact of specific N additions on a specific crop, and did not include any experimentation into the most appropriate and efficient harvesting, handling, storing and application methods of PMGMs.

## 2. Literature Review

### 2.1 The Nitrogen Cycle

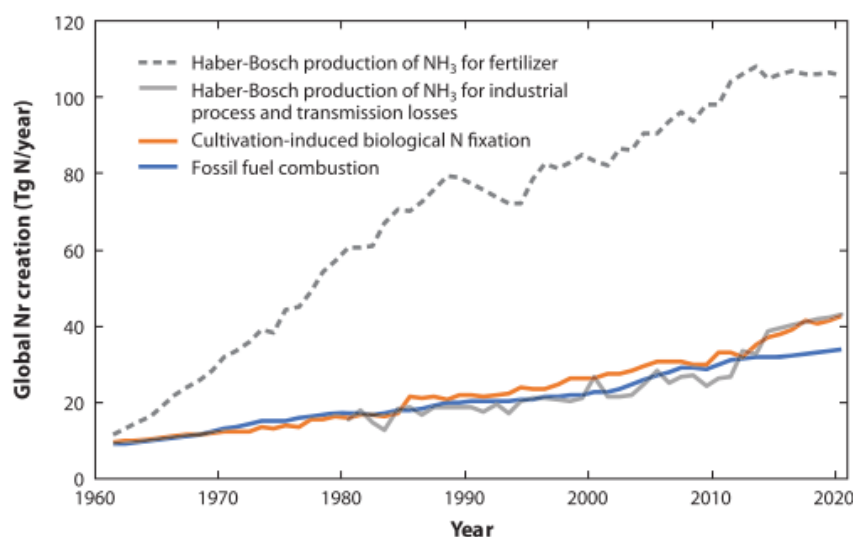
The atmosphere consists of 78% stable  $N_2$  (dinitrogen) which needs to be transformed, “fixed”, into a reactive form to be available to plants (Galloway, Bleeker and Erisman, 2021). Plants mainly take up N in its mineral form, specifically  $NH_4^+$  (ammonium) and  $NO_3^-$  (nitrate), which are created through biological N fixation, chemical N fertiliser production and through atmospheric returns (Cameron, Di and Moir, 2013; Chen *et al.*, 2014; Mahmud *et al.*, 2021) (Fig. 1). Recent research also indicates that plants are able to take up N in an organic form, but are often outcompeted by soil microorganisms (Bloom, 2015; Farzadfar, Knight and Congreves, 2021).



**Figure 1** The nitrogen cycle (Cameron, Di and Moir, 2013, p. 146), depicting the main mineral N pathways in an agricultural system where mineral fertilisers, biological N fixation including animal manures, and atmospheric returns introduce N to the agricultural system. Through a microbial process of mineralisation and immobilisation, the N becomes available for plants to take up, but is also subject to substantial losses through leaching and volatilisation.

Of the ways in which reactive N is introduced into the agricultural system, mineral fertiliser is clearly dominant. In 2020, the global anthropogenic introduction of reactive N was estimated to be 226 Tg, of which approximately 106 Tg came from Haber-Bosch production of  $NH_3$  (ammonia) fertiliser, and an additional 43 Tg from transmission losses and through Haber-Bosch production of  $NH_3$  for industry, compared to 43 Tg that was fixed through agricultural plants (the remaining 34 Tg

was a by-product from fossil fuel combustion) (Galloway, Bleeker and Erisman, 2021) (Fig. 2). In comparison, natural terrestrial ecosystems biologically fix approximately 58-128 Tg N per year (do Vale Barreto Figueiredo *et al.*, 2013; Fowler *et al.*, 2013; Soumare *et al.*, 2020; Galloway, Bleeker and Erisman, 2021). The use of reactive N in agriculture is thus highly dependent on the fossil fuel-based process of mineral fertiliser production and is reliant on reactive N levels beyond what natural ecosystems can provide.



**Figure 2** Human created reactive N globally (Galloway, Bleeker, Erisman, 2021, p. 258). Haber-Bosch produced fertiliser creates 106 Tg N yr<sup>-1</sup>, compared to Haber-Bosch production for industrial processes and transmission losses (43 Tg N yr<sup>-1</sup>), cultivation-induced biological N fixation (43 Tg N yr<sup>-1</sup>) and fossil fuel combustion (34 Tg N yr<sup>-1</sup>).

Globally, 50-55% of the N in fertilisers is lost to the environment (Zhang *et al.*, 2015; Houlton *et al.*, 2019; Martínez-Dalmau, Berbel and Ordóñez-Fernández, 2021; Rosa and Gabrielli, 2023). Mineral and organic fertilisers alike are subject to volatilisation (the loss of N at the soil surface through gaseous transformations) and leaching (the loss of N in runoff water) (Cameron, Di and Moir, 2013; Battye, Aneja and Schlesinger, 2017; Houlton *et al.*, 2019; Carr *et al.*, 2020) (Fig. 1).

### 2.1.1 Biological N fixation

N is naturally fixed in ecosystems by N-fixing species of microorganisms, independently or in association or symbiosis with plants (Peoples, Herridge and Ladha, 1995; Bloom, 2015; Soumare *et al.*, 2020). Through photosynthesis, plants gain C that is made available to the N-fixing microorganisms who use the energy to break the triple bond in the atmospheric N<sub>2</sub> and reduce it to NH<sub>3</sub> (ammonia), which in association with water, then becomes NH<sub>4</sub><sup>+</sup> (ammonium) and plant available (Whalen and Sampedro, 2010; Soumare *et al.*, 2020). The N fixers are bacteria, archaea and cyanobacteria, with Rhizobium bacteria which operate in symbiosis with legumes (Fabaceae family) most commonly used in agriculture (Soumare *et al.*, 2020).

The fixed N benefits the plant directly and its potential crop, such as N and protein rich seeds, peas and beans (Bloom, 2015; Batty, Aneja and Schlesinger, 2017). The biologically fixed N becomes available to other plants when the leguminous plants decompose or through the application of manure from livestock that has grazed the legumes (Carr *et al.*, 2020). Additionally, crops can access N when intercropped with legumes as the fixed N is transferred through the rhizosphere (Jensen, Carlsson and Hauggaard-Nielsen, 2020; Tian *et al.*, 2021; Zhao *et al.*, 2022). Biological N fixation is not subjected to N loss in that the N fixed is used by the plant directly, but the N can still be lost in consequent plant residue and manure break down (Soumare *et al.*, 2020).

N can also be fixed in the atmosphere by lightning, which leads to free hydrogen and oxygen atoms and the dinitrogen gas ( $N_2$ ) forming nitric acid ( $HNO_3$ ) (Bloom, 2015). This enters the soil system with rainfall and becomes nitrate ( $NO_3^-$ ) and part of the N cycle (Fig. 1).

2.1.2 Decomposition of organic material and mineralisation of organic N  
N fixed biologically enters the wider N cycle as the N fixing bacteria and associated plants break down in the soil. Soil macro and mesofauna start the decomposition process by consuming and breaking down the material into smaller pieces and mixing it with the soil, providing microfauna and subsequently microbial life and enzymes with extensive surface area for further decomposition (Whalen, 2014).

Organic N is released as extracellular enzymes break down the organic compounds (polymers) which can then be consumed by microbial cells to access the carbon (C) for respiration and growth (Crews and Peoples, 2005; Geisseler *et al.*, 2010). The N is often rapidly consumed by microbes and immobilised, until microbial death leads to remineralisation (Chen *et al.*, 2014). In cases where there is more N than microbial demand, which is limited by the amount of C available, mineral N becomes available in the soil as  $NH_4^+$  (Crews and Peoples, 2005; Whalen, 2014). The rate at which organic N is mineralised is therefore largely impacted by the C:N ratio of the decomposing species, with N typically being immobilised in microbial biomass if the C:N ratio is higher than 15 to 20:1 (Gaskell and Smith, 2007; Whalen and Sampedro, 2010). Over time, microbial respiration means a lowering of the C:N ratio in the soil and eventually leading to excess N, which is mineralised (Chen *et al.*, 2014).

Soil properties and the forms of C and N also influence mineralisation rates (Crews and Peoples, 2005; Chen *et al.*, 2014). Secondary metabolites, a broad group of chemical compounds produced by plants to handle biotic and abiotic strains, include phenolic compounds and lignin which have been shown to correlate with decomposition rates and the release of N (Chomel *et al.*, 2016). This extra resistance to decomposition occurs as complex resistant compounds are formed, often with toxic properties inhibiting fungi, bacteria and extracellular enzymes.

Other factors influencing decomposition include the location and placement of the plant matter. There is a greater abundance of decomposer organisms in the soil than on its surface, and buried leaf litter has been shown to decompose significantly

faster if buried, compared to being left on the soil surface (Whalen, 2014). Leaf litter has also been observed to degrade faster if decomposing close to its plant of origin, the so-called “home field advantage”. This is thought to be caused by the adaptation of the microbial population to the specific nature of the local leaf litter, such as the specific C:N ratios and secondary metabolites (Ayres *et al.*, 2009; Chomel *et al.*, 2016).

### 2.1.3 Mineral N fertiliser production

As the N levels required for high crop production are not met by biological fixation, the current agricultural system relies on supplementing the soil with reactive N through chemically produced fertilisers, often in the form of  $\text{NH}_4\text{NO}_3$  (ammonium nitrate) (Fageria and Baligar, 2005; Hirsch and Mauchline, 2015; Abdalla *et al.*, 2019). The production of such nitrogen fertiliser is done predominantly through the Haber-Bosch process, requiring pure hydrogen which is obtained from fossil fuels by removing  $\text{CO}_2$  through steam methane reforming and water-gas shift (Rosa and Gabrielli, 2023). This process both requires fossil fuels as feedstock and as energy input, which emits  $\text{CO}_2$  into the atmosphere, contributing substantially to the overall climate impact of the food system (Houlton *et al.*, 2019; Rosa and Gabrielli, 2023).

While the Haber-Bosch process is predominantly used in producing mineral N fertiliser, proposed alternatives/alterations to reduce GHG emissions include carbon capture and storage of the  $\text{CO}_2$  produced and emitted; electrification of the process and using electricity as feedstock to produce hydrogen through water electrolysis; or using biomass to supply both the fuel and feedstock, where biomass gasification supplies the hydrogen (Rosa and Gabrielli, 2023). Approximately 2 tonnes dry biomass is required to produce 1 tonne  $\text{NH}_3$ , having a significant impact on land use and ecosystems (Rosa and Gabrielli, 2023). These alternative routes are additionally typically more expensive than conventional fossil fuel based methods (IEA (International Energy Agency), 2021). Additionally, such technologies reducing the emissions to near or net-zero are not yet available at commercial scale (IEA (International Energy Agency), 2021).

### 2.1.4 Volatilisation

Volatilisation is the process through which  $\text{NH}_3$  is lost to the atmosphere in a gaseous form, one of the major N losses from agricultural soil (Cameron, Di and Moir, 2013; Mahmud *et al.*, 2021). This volatilisation typically happens immediately as fertilisers are applied (Mahmud *et al.*, 2021) and is particularly large in soils with high pH and/or with urea or urine application, as well as conditions with low soil moisture (Cameron, Di and Moir, 2013). It is also influenced by soil and air temperature (McGarry *et al.*, 1987; Mahmud *et al.*, 2021). In the atmosphere,  $\text{NH}_3$  is highly problematic as it can form inorganic aerosols, contribute to acid rain and eutrophication of waterways and be an indirect cause of  $\text{N}_2\text{O}$  emissions (Sutton *et al.*, 2008; Cameron, Di and Moir, 2013).

### 2.1.5 Nitrification and denitrification

N fertiliser applied is subjected to the simultaneous processes of nitrification and denitrification (Fig. 1. See more detail in Fig. 3). The aerobic conditions of nitrification enable microbes to convert  $\text{NH}_4^+$  to hydroxylamine ( $\text{NH}_2\text{OH}$ ), which becomes  $\text{NOH}$  and then  $\text{NO}_2^-$  (nitrite), which rapidly oxidises to  $\text{NO}_3^-$  (nitrate) (Cameron, Di and Moir, 2013). At both the  $\text{NH}_2\text{OH}$  and  $\text{NO}_2^-$  stages,  $\text{N}_2\text{O}$  (nitrous oxide) is produced (Cameron, Di and Moir, 2013). The anaerobic process of denitrification generates the transformation of  $\text{NO}_3^-$  or  $\text{NO}$  to  $\text{N}_2$  or  $\text{N}_2\text{O}$  (Mosier *et al.*, 1998; Mahmud *et al.*, 2021).

Agriculture is the largest anthropogenic source of  $\text{N}_2\text{O}$ , which is in particular created during the initial application of N fertiliser (Mahmud *et al.*, 2021).  $\text{N}_2\text{O}$  has a large warming potential, about 300 times that of  $\text{CO}_2$  (Cameron, Di and Moir, 2013), remains in the atmosphere for a long time (120 years) and causes ozone depletion (IPCC, 2007; Mahmud *et al.*, 2021).

### 2.1.6 Leaching

The  $\text{NO}_3^-$  produced during nitrification is anionic in nature, which makes it mobile in the soil and means that it can be washed away from the soil, or leach, especially in coarse well-drained soil (Mahmud *et al.*, 2021). The rate and amount of leaching is affected by rain and irrigation, as well as temperature, where colder temperatures entail greater precipitation and slower plant N uptake and higher leaching (Ahmed *et al.*, 2017; Mahmud *et al.*, 2021). Leaching tends to be greatest in sandy soils and for horticultural crops as these have higher fertilisation rates, shallower root systems, shorter growing seasons and are cultivated more frequently than arable crops (Cameron, Di and Moir, 2013).

Leaching also occurs in organic agricultural systems where  $\text{NO}_3^-$  concentrations from decomposing soil amendments are higher than plant uptake, with levels of N loss to leaching comparable to those seen in mineral fertiliser use (Stopes *et al.*, 2002). Largest leaching losses have been recorded after grass-clover leys, as organic N is mineralised but there is no plant demand (Andrews *et al.*, 2007; Cameron, Di and Moir, 2013). Deeper rooting crops such as alfalfa reduce leaching risk from the soil profile compared to shallow rooting crops such as potatoes (Cameron, Di and Moir, 2013). Leached  $\text{NO}_3^-$  enters waterways where it deteriorates water quality and leads to eutrophication and algal bloom, impacting environmental and human health (Cameron, Di and Moir, 2013; Mahmud *et al.*, 2021).

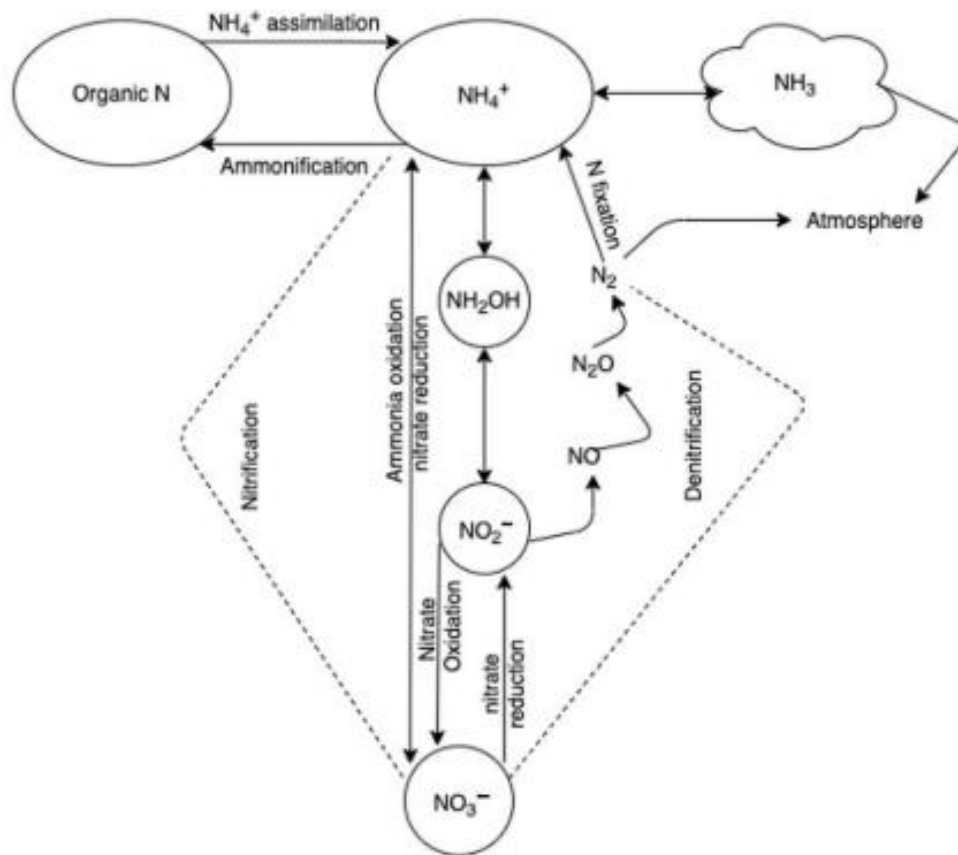


Figure 3 Details of the major processes of the N cycle in the soil (Mahmud et al, 2021, p.4).

## 2.2 Nitrogen Use Efficiency

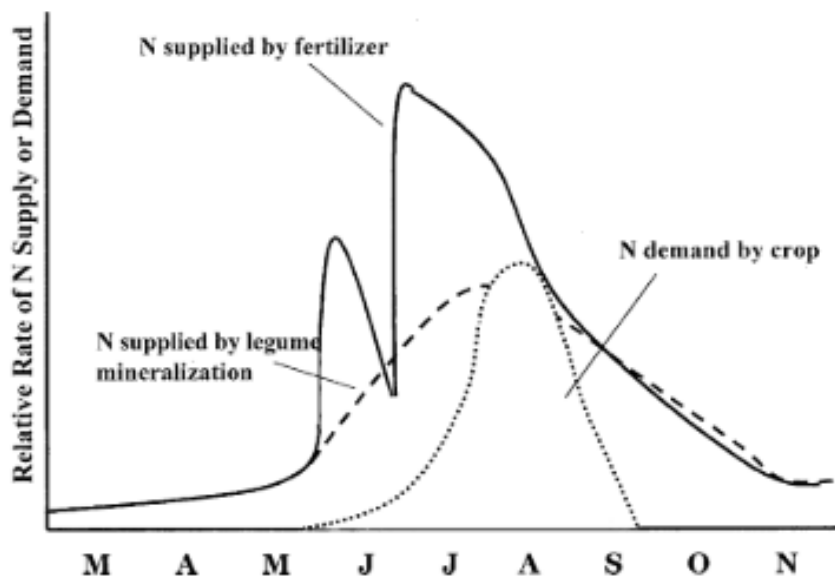
Nitrogen use efficiency (NUE) is a broad term that refers to the proportion of added N which is taken up by the crop and the capacity of the plants to use the N (Salim and Raza, 2020; Martínez-Dalmau, Berbel and Ordóñez-Fernández, 2021). It informs management practices, which need to balance the productivity of the crop with the NUE – NUE is highest in situations where there is low nutrient supply, and decreases as excess availability of nutrients increases (Salim and Raza, 2020).

Current NUE at a global scale is around 50% on average, meaning that about half of the mineral N fertiliser used is lost to the environment (Salim and Raza, 2020; Martínez-Dalmau, Berbel and Ordóñez-Fernández, 2021; Rosa and Gabrielli, 2023). It is essential to improve NUE as otherwise decreased N input required to meet climate goals may lead to increased food insecurity (Chang *et al.*, 2021).

To improve NUE, precision agriculture techniques have been developed where appropriately sourced fertiliser is applied in smaller and more precise doses at the time and in the location that the plants need it (Salim and Raza, 2020; Martínez-Dalmau, Berbel and Ordóñez-Fernández, 2021).

### 2.2.1 Synchrony

When N fertiliser application rates are matched to plant demand, there is very little residual N from the fertiliser in the soil profile left after harvest, reducing the risk of leaching (Jenkinson, 2001; Cameron, Di and Moir, 2013). This can be described as synchronising supply with demand (Crews and Peoples, 2005). Asynchrony thus occurs in the absence of N or when excessive N is supplied, but it also tends to occur after crop harvest or the incorporation of a legume crop (Crews and Peoples, 2005) (Fig. 4). It is difficult to achieve synchrony when working with biologically fixed N, such as that fixed by green manures, as there is no control over when the N is released in the soil as the manure breaks down (Crews and Peoples, 2005; Carr *et al.*, 2020).



**Figure 4** Issues with synchrony between crop demand and supply by either split application of mineral fertiliser or legume residue decomposition and mineralisation (Crews and Peoples, 2005, p. 113).

### 2.3 Green manures

Green manures are plants grown instead of a cash crop in order to increase the N available for subsequent crops through biological fixation (Cherr, Scholberg and McSorley, 2006; Blanco-Canqui *et al.*, 2015; Carr *et al.*, 2020). They are then tilled into the soil where they release the N that has been fixed or stored during the growth period (Abdalla *et al.*, 2019; Ferrara *et al.*, 2021). No-till methods of terminating the green manure growth include using herbicides, using roller-crimper machinery, or by letting it freeze in cold winter temperatures (Halde, Gulden and Entz, 2014; Frascioni *et al.*, 2019; Alonso-Ayuso *et al.*, 2020). Green manures are commonly a mix between N-fixing legume species such as red clover (*Trifolium pratense*), white clover (*Trifolium repens*), hairy vetch (*Vicia villosa*) and alfalfa (*Medicago sativa*), and non-N fixing species such as perennial ryegrass (*Lolium perenne*) and cocksfoot (*Dactylis glomerata*) (Cherr, Scholberg and McSorley, 2006; Carr *et al.*, 2020).

Cover crops differ from green manures in that they are crops sown on otherwise fallow land. They are often grown over winter to reduce soil erosion and N leaching



by taking up and storing the available  $\text{NO}_3^-$  (Dabney *et al.*, 2010; Cameron, Di and Moir, 2013; Blanco-Canqui *et al.*, 2015).

Green manure N fixation rates vary, and depend on many factors such as temperature and soil, rendering large ranges of estimation (Cuttle, Shepherd and Goodlass, 2003; Carr *et al.*, 2020). Crimson clover has been shown to fix between 56 and 170 kg N ha<sup>-1</sup> (Dabney *et al.*, 2010). White clover is estimated to fix 100-200 kg N ha<sup>-1</sup> in the UK and red clover is estimated to fix slightly more (Cuttle, Shepherd and Goodlass, 2003).

### 2.3.1 Land use issue

Setting aside fields for a growing season or more to grow green manures lowers the amount of available arable land for crop production, with up to 66% of the acreage set aside in green manures in organic farms (Høgh-Jensen and Schjoerring, 1997; Cuttle, Shepherd and Goodlass, 2003). This substantially lowers the amount of available land for cash crop production, meaning that any increase in organic agriculture, whilst decreasing reliance on and GHG emissions from fossil fuels, would necessitate an increase in land use, infringing on natural habitats and the ecosystem services they provide (Balmford, Green and Phalan, 2015). The overall efficacy of the agricultural system is dependent on many factors, ranging from N input and uptake to food waste to diet choices (Houlton *et al.*, 2019), but transitioning the system to relying on biologically fixed N through green manures would necessitate more land than is required with mineral N fertilisers, calling into question future food security (Smith *et al.*, 2018).

### 2.3.2 Green manure NUE and C:N ratios

The management practices associated with green manures, incorporating them into the soil and leaving them to decompose in situ, mean that it is difficult to synchronise the N supply released during decomposition with the demands of subsequent crops (Crews and Peoples, 2005; Carr *et al.*, 2020). The C:N ratio of a legume green manure such as red clover is typically around 10-15:1, meaning that the decomposition happens relatively quickly and the associated release of N tends to happen within 4-8 weeks (Gaskell and Smith, 2007; Carr *et al.*, 2020). This means that green manures often have a lower or equivalent NUE to mineral fertiliser (Crews and Peoples, 2005).

### 2.3.3 Green Manures N loss

Green manures are subject to the same risks of N loss through volatilisation and leaching as other N additions to the soil. The range of recorded N loss through volatilisation varies greatly, from 0.1% of clover N (Harper *et al.*, 1995) to 17-39% (Larsson *et al.*, 1998) (Table 1). Lower C:N ratios in the decomposing green manure entail higher volatilisation rates (de Ruijter, Huijsmans and Rutgers, 2010). Incorporating the green manure into the soil has been shown to decrease the volatilisation rate (de Ruijter and Huijsmans, 2019).

**Table 1** N loss pathways and rates of green manures

<b>N LOSS PATHWAY</b>	<b>RATE OF N LOSS</b>	<b>GREEN MANURE SPECIES</b>	<b>REFERENCE</b>
<b>VOLATILISATION</b>	0.1%	Clover	(Harper <i>et al.</i> , 1995)
	0.31%	Faba bean	(Ferrara <i>et al.</i> , 2021)
	0.3-0.5%	Pea residue	(Engel, Jones and Wallander, 2013)
	1.2%	Cauliflower residue	(Nett <i>et al.</i> , 2016)
	2.7-5.6%	Red clover	(Båth <i>et al.</i> , 2006)
	4.5-6%	Broadbean	(Rana and Mastrorilli, 1998)
	5%	Lentil	(Janzen and McGinn, 1991)
	5%	Yellow mustard	(de Ruijter, Huijsmans and Rutgers, 2010)
	16%	Grass	(Larsson <i>et al.</i> , 1998)
	17%	Alfalfa	(Larsson <i>et al.</i> , 1998)
<b>LEACHING</b>	39%	Grass (high-N)	
	58 %	Hairy vetch mulch	(Campiglia <i>et al.</i> , 2011)
	20-60%	Leek & broccoli residues	(De Ruijter, Ten Berge and Smit, 2010)
	27-39%	Clover rye grass	(Høgh-Jensen and Schjoerring, 1997)
	6-16%	Red clover	(Bergström and Kirchmann, 2004)

Systems using green manures and/or incorporating plant residues into the soil have been shown to be at risk of high leaching rates of  $\text{NO}_3^-$ , and can have similar leaching loss rates to systems using mineral fertiliser (Stopes *et al.*, 2002; Bergström and Kirchmann, 2004; Campiglia *et al.*, 2011) (Table 1). Green manures are also subject to  $\text{N}_2\text{O}$  emissions, where low C:N ratio additions are at the greatest risk as excess N is subject to denitrification (Möller and Stinner, 2009).  $\text{N}_2\text{O}$  emission rates of clover green manures have been recorded between 0.5 and 6% of N applied (IPCC, 2019; Ward, 2020)

#### 2.3.4 Mobile green manures

To address the issues of synchronising N supply with crop demand in green manures, mobile green manures (“cut and carry”) have been suggested. This is when green manures are grown, harvested and added to fields when needed depending on crop demand (Crews and Peoples, 2005; Sorensen and Grevsen, 2016; Gatsios *et al.*, 2021). This can be done with fresh growth, or the green manures can be harvested, processed and stored before application, e.g. through composting, anaerobic digestion or ensiling (Sørensen *et al.*, 2013). Trials have shown that the NUE is improved through such processing, although care must be taken to not incur  $\text{N}_2\text{O}$  and  $\text{NH}_3$  emissions during the process (Möller and Stinner, 2009; Benke *et al.*, 2017; Gatsios *et al.*, 2021).

#### 2.4 Perennial Mobile Green Manures

Using perennial green manures has been suggested as a way to overcome both the land use and synchrony issues of conventional green manures (Ward, Chadwick and Hill, 2023). Woody perennial nitrogen fixing species, such as alder, can be used in intercropping or alley cropping systems, where crops are grown between rows of trees or shrubs that take up excess N and prevent leaching, whilst prunings can be applied to the soil as a green manure (Haggard *et al.*, 1993; Crews and Peoples, 2005).

This is most common in tropical agroforestry systems, where the fixed N is transferred to crops through the decomposition of leaf litter and prunings, directly root to root or through mycorrhizal networks (Munroe and Isaac, 2014). The amount of N taken up by the crop is however small, typically 10-30% of the N provided by decomposing tree prunings, with the rest of the N becoming immobilised in the soil or taken up by the tree again (Sileshi, Mafongoya and Nath, 2020). This highlights the importance of understanding the C:N ratio, lignin and polyphenol contents influencing the decomposition of leaves and prunings and mineralisation of the N for best application time and method.

A recent study has examined using perennial mobile green manures (PMGMs) in a temperate climate, where the green manures are grown in permanent areas of agricultural or marginal land and added to cropland to meet N demand (Ward, Chadwick and Hill, 2023). The study examined Alder (*Alnus glutinosa*), Gorse (*Ulex europaeus*) and Gunnera (*Gunnera tinctoria*) compared with red clover as a conventional green manure. Cuttings from the plants were chopped and added to ryegrass (*Lolium perenne*) in a greenhouse experiment, where PMGMs yielded similar or higher biomass than clover. In a two-year field experiment, it was shown that the crop N uptake from PMGMs increased in the second season and have the possibility to improve NUE compared to conventional green manures (Ward, Chadwick and Hill, 2023).

Such an approach enables the use of perennial N fixing plants that are able to grow on marginal exposed or wet land, and thus the N fixing in the system does not require the setting aside of arable and otherwise crop producing fields. Additionally, the perennial nature of the green manure stands has additional ecosystem benefits such a C sequestration, improved soil health and providing wildlife habitat (Munroe and Isaac, 2014; Sileshi, Mafongoya and Nath, 2020; Ward, Chadwick and Hill, 2023).

This study continues in this line of research. Alder, Scotch broom (*Cytisus scoparius*) and gorse have been chosen as perennial green manures to be investigated. Alder is a tree native to the UK which typically grows in wet or flooded areas. It can be coppiced and fixes N through a symbiotic relationship with the *Frankia* bacteria (Claessens *et al.*, 2010). Gorse and Scotch broom are also native to the UK and tend to grow on marginal and exposed soils where they fix N in symbiosis with the *Rhizobia* bacteria (Paynter *et al.*, 2000; Atlan *et al.*, 2015). They are often considered invasive as they proliferate quickly, and much of the scientific literature has examined appropriate ways to remove them from farmland and nature reserves (Broadfield and McHenry, 2019). Gorse and broom have further been studied in regards to weed control, with studies registering a phytotoxic effect due to phenolic compounds in the leaves, leading to decreased weed germination and growth in soils with incorporated gorse or broom leaf litter, without any impact on microbial life or crop (Pardo-Muras, Puig and Pedrol, 2019, 2022; Pardo-Muras *et al.*, 2020).

### 3. Methodology

#### 3.1 Pot experiments

Studying plants and their responses to environmental conditions in their natural habitat is complex and comes with a range of logistical and analytical constraints (Gibson et al., 1999). Pot experiments on the other hand, are carried out in settings where variables such as soil, pathogens, water, light etc can be controlled (Poorter, Fiorani, et al., 2012). This limitation of random biotic or abiotic factors means direct and precise measurements can be obtained, generating repeatable experiments which can be subjected to statistical analysis (Hairston, 1989).

Pot experiments are however limited in having low degrees of realism, i.e. factors are regulated unlike natural communities, and restricted over temporal and spatial scales (Diamond, 1986; Scheiner and Gurevitch, 2001). There can also be inadvertent constraints, such as inadequate pot size limiting the rooting possibilities, especially in longer running experiments with perennials (Poorter, Bühler, et al., 2012; Kawaletz et al., 2014).

Agroecosystems, the systems of organisms, physical environments, and structures where crop production is the primary function, are complex and impacted by a range of factors including soil, climate, farming practices, socio-cultural factors etc (Drinkwater, 2002). The development of sustainable best practice in agroecosystems necessitates a combination of controlled pot and field with whole-systems research approaches (Drinkwater, 2002).

Conducting a pot experiment outside in an experimental garden brings it closer to conditions the crop would face in the field, and whilst variations in light, temperature and water cannot be controlled to the extent that is possible in a greenhouse, this level of control is only necessitated if the effects of those factors are being studied (Poorter, Fiorani, et al., 2012). To account for factors such as prevailing wind and sun exposure, randomised block designs can be used, in which treatments are randomised in blocks that are arranged so that variation within blocks is minimised, ensuring an equal exposure to the factors outwith experimental control (Poorter, Fiorani, et al., 2012).

#### 3.2 Choice of crop, green manures and soil

In this study, “Red Russian” Kale (*Brassica napus* var *Pabularia*) was grown outside in pots. There are several varieties of kale, and it is a popular crop worldwide due to its ability to grow in different conditions and its high levels of beneficial nutrients (Migliozzi et al., 2015; Satheesh and Workneh Fanta, 2020). It was suitable for the experiment as it, despite being an annual, has a long season and is known to germinate and grow well in the climatic zone of the experiment (Yoder and Davis, 2020; Park et al., 2022). It was further selected for field trials of perennial green manures, and it was deemed prudent to study the same crop in different experimental set ups to enable complementary data as discussed by Drinkwater (2002).

The perennial green manures chosen for this experiment were alder (*Alnus glutinosa*), Scotch broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*). These are native to the area in which the experiment was carried out, often growing prolifically on marginal land (Paynter *et al.*, 2000; Claessens *et al.*, 2010; Atlan *et al.*, 2015). Previous studies in perennial green manures have examined alder and gorse (Ward, Chadwick and Hill, 2023). To date there have not been any studies considering the nitrogen efficiency of broom as a perennial green manure. Perennial green manures were compared to a green manure already in general use, red clover (*Trifolium pratense*), with one treatment consisting only of leaf and one consisting of leaf and root. Previous perennial green manure studies have made this comparison, but only used the clover leaf (Ward, Chadwick and Hill, 2023). Clover roots have been included in this study to be more representative of field conditions.

The performance and suitability of green manures is impacted by the soil type, with sandy soils being especially prone to N leaching loss if there is not complete synchrony between green manure N release through decomposition and crop N demand (Cherr, Scholberg and McSorley, 2006). This mandates an investigation in sandy soils in particular.

### 3.3 Assessing nitrogen uptake

Organic amendments to soil supplies nutrients such as N, add organic matter to the soil, and does not have the embodied CO<sub>2</sub> of mineral fertiliser (Cherr, Scholberg and McSorley, 2006; Blanco-Canqui *et al.*, 2015). The crop uptake of N from organic amendments such as green manures depends on the structure and decomposition rates of the green manure as well as the soil, where higher C:N values tend to indicate a slower availability of N for crops (Chen *et al.*, 2014; Whalen, 2014). The efficiency of N recovery can be tested in pot and field experiments where a known amount of N is supplied in the organic amendment and subsequent N content of crop growth is analysed (Ovsthus *et al.*, 2017; De Notaris *et al.*, 2018). Biomass growth is roughly correlated to crop nutrient demands (Whalen, 2014), so root and plant biomass levels were assumed to be an adequate indicator of N uptake (Poorter, Fiorani, *et al.*, 2012; Yoder and Davis, 2020), as the experiment was carried out on a low budget and without access to laboratory equipment.

Root morphology is dependent on many factors such as soil texture and climatic conditions, but is also influenced by nutrient availability (Freschet *et al.*, 2021). Root branching is especially sensitive to N availability in poor soils (Freschet *et al.*, 2018). Root ecology is a large field of study with varying terms for root traits, a large number of methodologies for measurement, and challenges regarding accounting for variations in root systems that occur over time and space (Freschet *et al.*, 2021). Cornelissen *et al.* (2003) have suggested a few straightforward standardised methods, including specific root length (the ratio of root length to mass), root depth distribution (vertical distribution of root biomass in the soil) and nutrient uptake strategy. Root morphology often contains the easiest traits to measure, and additionally include root diameter and root tissue density, whilst root system architecture can be measured by root branching density (the number of laterals on a given length of root) (Freschet *et al.*, 2021). All these measurement methods require

laboratory and microscopic equipment. The methodology of any experiment measuring roots must consider the balance between extensive root harvesting and processing, the necessary labour needed for this, and damage to plants and root systems (Freschet *et al.*, 2021), which in the experiment for a master's level, meant that simplified measurements of root length, diameter and branching density were used.

Colour of crop leaves are a known indicator of chlorophyll and N content (Ali *et al.*, 2013) and colour charts are routinely used by rice and maize farmers to assess N availability and modify fertilizer applications (Varinderpal *et al.*, 2011; Brajendra Singh *et al.*, 2021). In the absence of similar leaf colour charts for other crops, low technology observations of leaf colour can be made using other colour references, such as Munsell colour charts (Munsell, 1971; Raese, Drake and Curry, 2007), which was the approach used in this study.

### 3.4 Decomposition assessments

Litterbags have been a standard methodology for assessing decomposition rates at the soil surface (Krishna and Mohan, 2017). Mesh bags are filled with fresh leaves and placed on the soil. The size of the mesh is selected to enable access of decomposing organisms while preventing particle loss from the decomposing material, with 1-2 mm mesh deemed most suitable (Robertson and Paul, 2000).

Litterbag methods have been criticised as the bag and the mesh size influence the activity of decomposing macrofauna and can have a significant impact on measured decomposition rates, as can the sample weight and placement of litterbags (Xie, 2020). However, they remain a low-tech way of assessing decomposition and so were a practical way to compare decomposition rates between treatments in this study.

### 3.5 Timeframe

Perennial green manures tend to be woodier species and thus have higher C:N ratios than annual more commonly used green manures. As this will impact the N release (Whalen, 2014), the effects of perennial green manures should not be expected to be seen in experiments with a short time frame. Other studies have started observing N release from gorse and alder around 120 days after application (Ward, Chadwick and Hill, 2023), giving an indication for the shortest possible time span of an experiment. If carried out over several growing seasons, the effect of slow nitrogen release would be accounted for more thoroughly (Ward, Chadwick and Hill, 2023). However, the experiment was limited to a growing season as it was an outside pot experiment of an annual crop (Poorter, Fiorani, *et al.*, 2012). This timeframe was also directed by the timings of the course studied.

### 3.6 Data analysis

Calculating the statistical significance of experiment results enables conclusions to be drawn that are representative of the characteristics of the whole group of study, despite observing only a smaller sample of individuals (Townend, 2013). When examining the difference between populations or treatments, the probability difference is expressed as the P-value, where  $P < 0.05$ , i.e. less than 5% chance of no

difference, is generally considered statistically significant (Quinn and Keough, 2002). Critiques point out that P-values only show how the data conforms to the assumptions of the test hypothesis but do not address any issues within the hypothesis itself (Greenland *et al.*, 2016) and that the magnitude of the P-value for so called significant results can vary beyond the 0.05 value (Berger and Sellke, 1987).

For the purposes of this study, carried out on a small scale and without laboratory equipment, the 0.05 significance level has been used in analysis of variance (ANOVA) with the typically conservative Bonferroni adjustment. This was to minimise the risk of Type 1 error (finding difference when there is none) when making multiple comparisons (Abdi, 2007).

Many statistical tests, including ANOVAs, are parametric and assume a normal distribution of data, where most values are close to the mean (Quinn and Keough, 2002). It is however common for size data of individually grown plants to be non-parametric, with many small and a few large plants (Poorter, Fiorani, *et al.*, 2012). In such cases, non-parametric tests can be used but normally generate less robust results (Townend, 2013). Alternatively, data transformation can be done to give non-parametric data a normal distribution, which can then be subjected to parametric tests (Townend, 2013).

## 4. Methods

### 4.1 Green Manure sourcing

Alder, gorse and broom were harvested on 29/05/2023 from Cullerne Garden, Findhorn, Moray (57°39'11.1"N, 3°35'48.0"W). Alder was harvested in the form of leaves and stems from established trees. Gorse and broom were harvested by cutting the outer 20cm of branches from bushes that were in full bloom. Clover was harvested from the same place on the following day, 30/05/2023, from a crop sown on 31/03/2023. Clover was cut at soil level and any other self-germinated species were discarded. Clover was also dug up to include root mass, other plant species were separated away and discarded, and plants were lightly shaken and blown on which was sufficient to remove any sandy soil remaining on the roots. All green manures were stored in plastic bags at 8°C for 11 and 10 days respectively until the experiment was set up. Samples of each green manure were sent to NRM laboratories to determine moisture, C and N contents (Table 2).

*Table 2 Properties of the green manures used in the experiment, values from NRM laboratories from a single measure of a representative sample*

Sample Reference		ALDER	BROOM	CLOVER	CLOVER AND ROOT	GORSE
Determinand	Unit	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Dry Matter	%	26.8	23.1	13.9	15.0	34.8
Total Nitrogen DUMAS	% w/w	3.52	3.63	3.83	3.45	2.75
Total Phosphorus	% w/w	0.33	0.36	0.53	0.51	0.19
Total Potassium	% w/w	1.20	1.61	2.90	2.55	0.94
Total Calcium	% w/w	0.93	0.46	2.45	2.11	0.42
Total Magnesium	% w/w	0.31	0.22	0.67	0.69	0.21
Total Sulphur	mg/kg	1955	1833	1750	1686	1569
Total Carbon	%	53.2	52.7	47.2	44.7	52.3
Carbon:Nitrogen Ratio	:1	15.1	14.5	12.3	13.0	19.0
Total Manganese	mg/kg	130	105	53.2	79.1	76.8
Total Copper	mg/kg	9.0	4.3	5.8	8.8	3.7
Total Zinc	mg/kg	60.5	79.7	37.7	46.7	66.6
Total Iron	mg/kg	60.4	82.1	80.5	583	104
Total Boron	mg/kg	17.3	19.4	28.4	25.8	18.7

### 4.2 Soil sourcing

Soil was collected on 30/05/2023 from a depth of 2-15cm from an unused field in Cullerne Garden, Findhorn, Moray (57°39'11.1"N, 3°35'48.0"W), which had been covered for 2 years. Prior to that, it had been farmed with organic amendments such as compost, manure and rock dust since 1975. Rudimentary soil tests were carried out indicating a friable structure and loamy sand texture. It was sieved through a course sieve ( $\leq 10$ mm) and stored in a cool shaded outside location for 10 days before being used in the experiment. Samples of the soil were sent to NRM laboratories to determine further properties (Table 3).



**Table 3** Soil properties, values from NRM laboratories from a single measure of a representative sample

SOIL PROPERTY	
PH	7.1
NO <sub>3</sub> <sup>-</sup> (MG N L <sup>-1</sup> )	24.26
NH <sub>4</sub> <sup>+</sup> (MG N L <sup>-1</sup> )	1.09
AVAILABLE P (MG L <sup>-1</sup> )	99.8
AVAILABLE K (MG L <sup>-1</sup> )	210
AVAILABLE MG (MG L <sup>-1</sup> )	215

### 4.3 Growth experiment set-up

The experiment was conducted in Findhorn, Moray (57°39'17.1"N, 3°35'16.8"W) and consisted of 7 treatments (including control) with 6 repetitions of each, giving a total of 42 pots which were set up 08-09/06/2023. The treatments were no addition (control) and 200 kg ha<sup>-1</sup> N equivalent of alder, broom, clover, clover+root, gorse and mineral fertiliser ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) (AN). Three litre pots measuring 16x16x20.5cm (Muddy Hands Ltd., Birmingham, UK) were filled with 1000g soil. Green manure treatments were cut into ≤4cm pieces and mixed thoroughly with 800g of soil, and then added on top of the 1000g of soil already in the pots. The fresh weight of each green manure treatment needed to obtain a 200 kg ha<sup>-1</sup> N equivalent was calculated using the N content, moisture content, and pot surface area. Control and AN pots were filled with a total of 1800g soil. The pots were placed outside on wooden pallets in a randomised block design in a netted cage and left for 20 days, following DEFRA guidance that cover crops and green manures should be tilled and left in the soil for at least three weeks prior to sowing a cash crop (DEFRA, no date).

On 29/06/2023, 6 seeds of Red Russian Kale (*Brassica napus* var *Pabularia*) per pot were sown at a depth of 10mm and covered with the soil in the pot. In order to minimise the effect of other nutrients than N on the crop growth, UK fertiliser guidelines for brassica crops were followed for P, K and Mg (AHDB, 2021). Based on the values in Table 3, this meant that 150 kg ha<sup>-1</sup> equivalent of K was added to each pot at the time of sowing. At this point, 200 kg ha<sup>-1</sup> equivalent of AN was also added to the 6 AN pots.

Pots were free draining and evenly watered in the event of hot and dry weather, as per standard crop treatment. The experiment ran for 138 days after sowing until 14/11/2023.

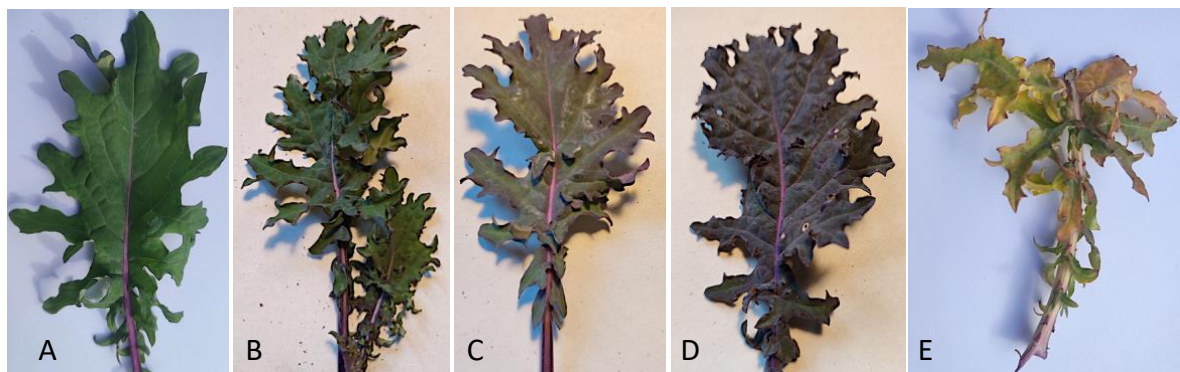
### 4.4 Decomposition experiment set-up

In addition to the growth experiment, a decomposition experiment of the green manures was carried out. On 13/06/2023, 10x12cm bags of 2mm mesh were filled with 4g wet weight of the alder, broom, clover, clover+root and gorse treatments respectively. 5 repetitions of each treatment were pinned in a randomised design to the sandy soil at the experiment site and left for 102 days. The bags were unattended for the duration of the experiment and were covered by leaves, soil and grass by the time they were harvested.

#### 4.5 Crop Sampling

Crop germination was recorded 25 days after sowing. Weed germination was recorded at the time of sowing and after 25 days, when wet weight, height, root length and species were also recorded. Species were identified using a weed ID guide (AHDB, 2016).

Crop height above soil level, as well as qualitative measures of sturdiness (ranked 1-5) and colour of the crops were recorded roughly every two weeks, 34, 49, 63, 78, 91, 109, 120 and 138 days after sowing. The colour was defined by determining the nearest resemblance to one of five selected colours using Munsell colour charts (Fig. 5).



**Figure 5** Colours of crop leaves were recorded by determining the closest resemblance to one of five colours (Munsell specification in brackets) A) Green (5G 2/4) B) Dark green (7.5G 3/6) C) Bluegreen (2.5BG 2/4) D) Purple (7.5P 1/4) E) Yellow (7.5GY 8/12)

34 days after sowing, all but the two largest plants in each pot were harvested, recording above ground height, weight and morphological features, as well as root length and weight. 82 days after sowing, again the smallest plant in each pot was harvested, leaving one plant left per pot, and recording above ground biomass height, weight, colour and sturdiness, as well as root weight, length and morphology. Root diameter was measured at a single spot 10mm below the soil level. The same data was recorded when the final plants were harvested 138 days after sowing (Table 4). All biomass was dried at 80°C for 24 hours and dry weight was also recorded.

The decomposition bags were taken up after 102 days, extraneous soil and plant matter was removed, and dry weight was recorded after drying for 24 hours at 80°C.

All samples were weighed on a balance accurate to 0.01g (Apex CE Specialists Ltd, Manchester, UK).

**Table 4** Main experiment timeline from sowing to final harvest, detailing timings of data observations

Date (2023)	June 29th	July 24th	Aug 2nd	Aug 17th	Aug 31st	Sep 15th	Sep 19th	Sep 28th	Oct 16th	Oct 27th	Nov 14th
Days after sowing	0	25	34	49	63	78	82	91	109	120	138
Seeds sown	x										
Weed Harvest - above and below ground growth data recorded	x	x									
Crop germination count		x									
Crop Harvest - above and below ground growth data recorded			x				x				x
Crop Above ground growth height, sturdiness and colour recorded			x	x	x	x		x	x	x	x

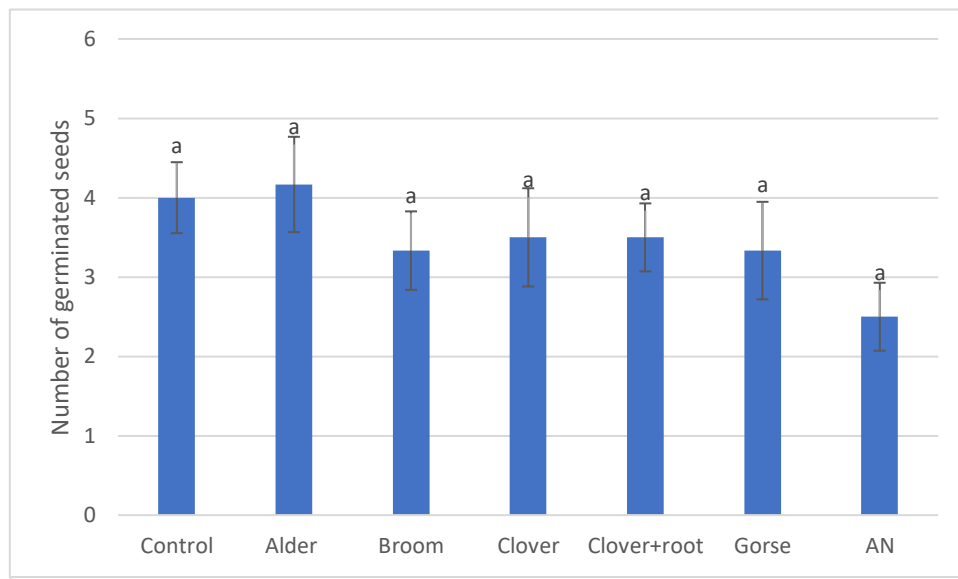
#### 4.6 Data analysis

Statistical analysis was performed using Microsoft Excel v. 16.16.27 (Microsoft, 2018). One-way analyses of variance (ANOVA) with Bonferroni post hoc tests were used to determine significant differences between treatments. A skewness test was performed to ascertain the distribution of the data and any non-parametric data was rank transferred before conducting the ANOVA.

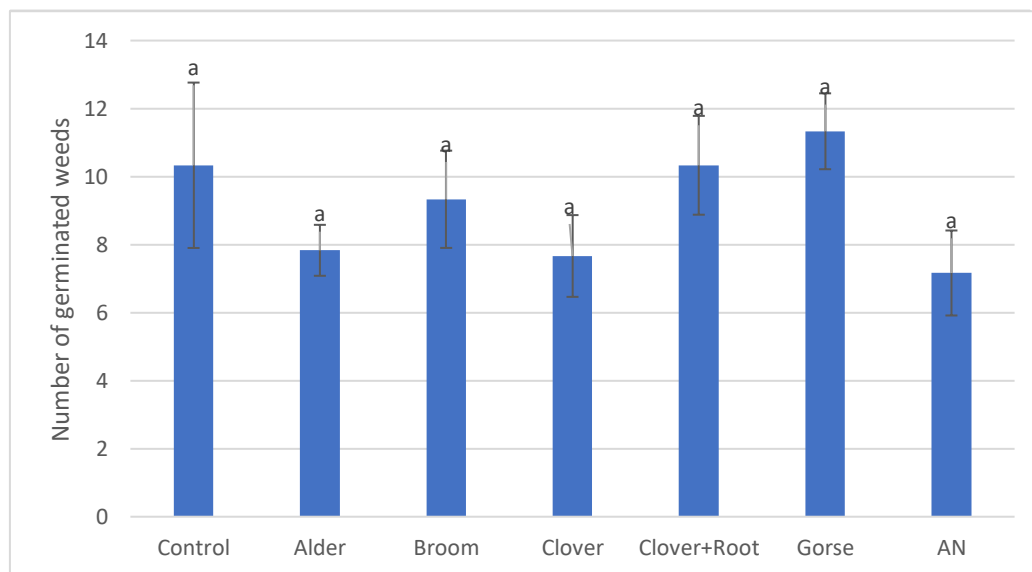
## 5. Results

### 5.1 Germination rates

On average, 3-4 crop seeds germinated out of the 6 sown in the green manure treatments. The AN treatment had a slightly lower average of 2.5 germinated seeds out of 6, but there was no significant difference ( $P>0.05$ ) between treatments (Fig. 6). There was no significant impact of treatment on self-germination of weeds recorded at 25 days after sowing, with averages ranging between 7 and 11 weeds per pot (Fig. 7).



**Figure 6** Number of seeds germinated 25 days after sowing, out of 6 sown per pot. Means  $\pm$  SEM ( $n=6$ ), no statistical difference at  $P<0.05$ .

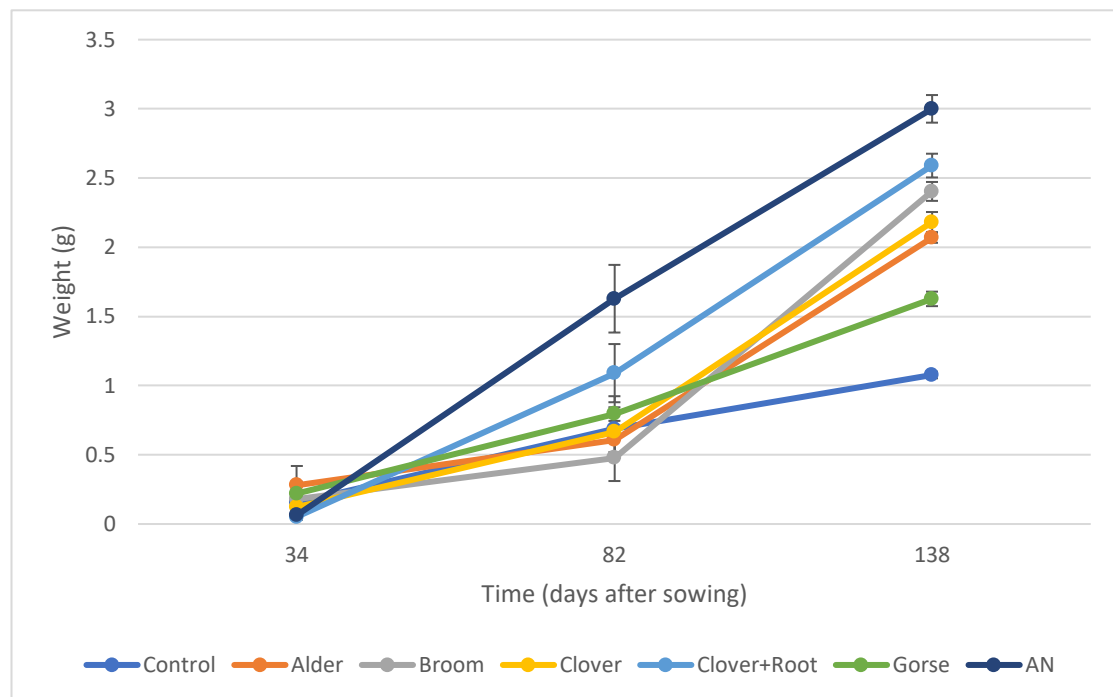


**Figure 7** Number of weed seeds that had self-germinated 25 days after sowing. Means  $\pm$  SEM ( $n=6$ ), no statistical difference at  $P<0.05$

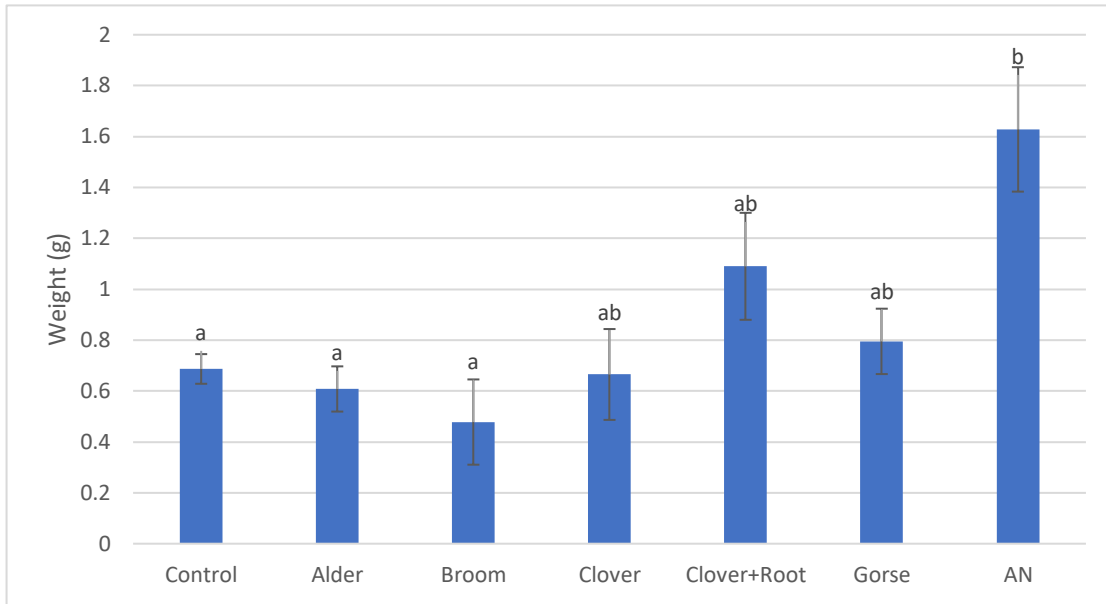
## 5.2 Above ground biomass of crop

Plants from each pot were destructively harvested and weighed at three occasions during the experiment. 34 days after sowing, there was no significant difference ( $P>0.05$ ) of the dry weight (ranging from 0.05 to 0.3 g) of crops between different treatments. Plants that had received AN treatment weighed the most in both following harvests, followed by the clover+root treatment (Fig. 8).

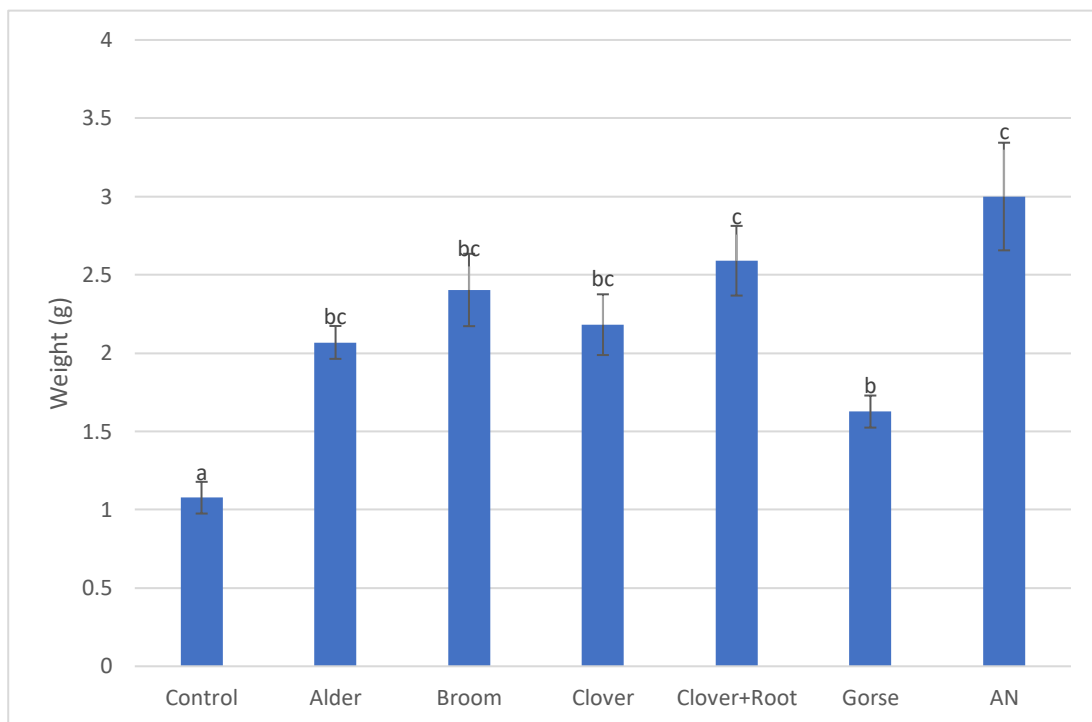
82 days after sowing, crops with the AN treatment had a mean dry weight of 1.63 g and had significantly ( $P<0.01$ ) more mass than plants with the control, alder and broom treatments, which weighed 0.69g, 0.61g and 0.48g respectively (Fig. 9). By 138 days after sowing, the control had a mean dry weight of 1.08g, which was significantly lower than all other treatments ( $P<0.01$ ). Additionally, the mean dry crop weight of the AN (3g) and clover+root (2.59g) treatments were significantly higher than the gorse (1.63g) treatment ( $P<0.05$ ), but there was no significant difference in weight between AN, clover+root, clover (2.18g), alder (2.07g) and broom (2.40g) treatments (Fig. 10).



**Figure 8** Dry weight of crop biomass above ground throughout the experiment. Means  $\pm$  SEM.

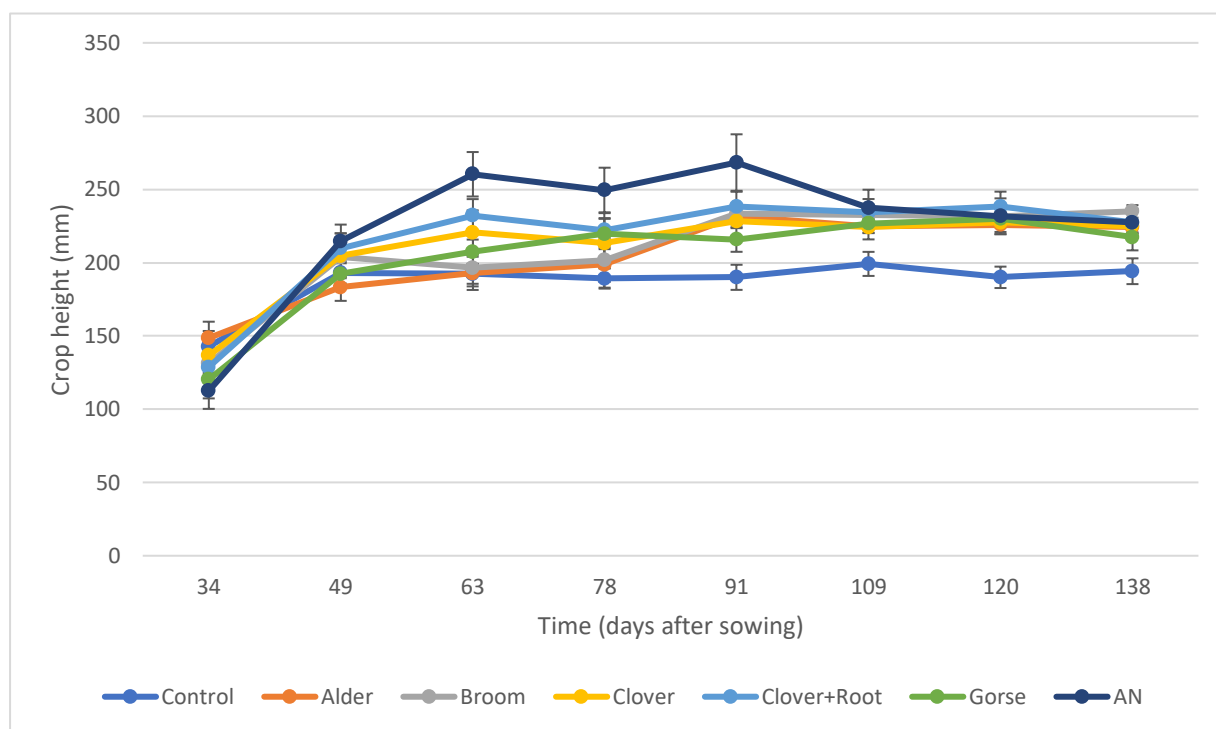


**Figure 9** Dry weight of crop biomass above ground 82 days after sowing. Means  $\pm$  SEM (n=6), letters indicate statistical significance at  $P < 0.05$



**Figure 10** Dry weight of crop biomass above ground 138 days after sowing. Means  $\pm$  SEM (n=6), letters indicate statistical significance at  $P < 0.05$

As the kale plants continuously dropped leaves and grew new ones, the height of the plants did not increase linearly throughout the season. Rather, the height can be seen as the ability to regenerate leaves of the same size. AN treated pots had the highest plants between approximately 50 and 100 days after sowing with average heights of 250-270mm. At the end of the experiment there was little difference in heights between treatments, with kale grown in all green manures measuring in the range of 215-235mm and the control measuring 194mm (Fig. 11). Significance varied throughout the experiment (see Table 5): after 63 days, AN treated plants measured 260mm on average and were significantly ( $P<0.01$ ) higher than the means of alder (193mm) and the control (192mm). After 78 days, AN treated plants were 250mm and significantly ( $P<0.01$ ) higher than the control (189mm). At the later measurements in the experiment, the AN treated plants decreased in height and instead alder, broom and later clover treatments registered significantly higher plants than the control with 232mm (alder) and 233mm (broom) at 91 days ( $P<0.01$ ) and 232mm (broom) and 228mm (clover) at 120 days ( $P<0.01$ ). After 138 days, only broom (235mm) was significantly higher than the control (194mm) ( $P<0.01$ ).



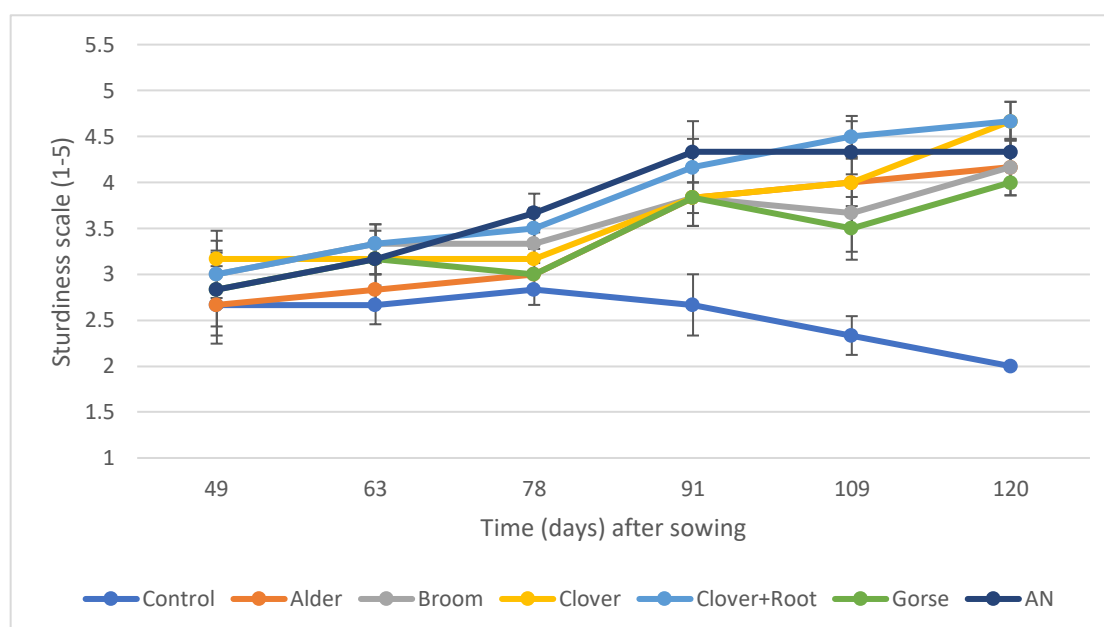
**Figure 11** Crop height (mm) above soil level throughout the experiment. Means  $\pm$  SEM.

**Table 5** Overview of significant differences ( $P < 0.01$ ) of crop heights (mm) between treatments throughout the experiment

Days after sowing	Treatment and height significantly ( $P < 0.01$ ) higher ...	... than (treatment and height)
63	AN: 260mm	Control: 192mm Alder: 193mm
78	AN: 250mm	Control: 189mm
91	Alder: 232mm Broom: 233mm	Control: 190mm
120	Broom: 232mm Clover: 228mm	Control: 190mm
138	Broom: 235mm	Control: 194mm

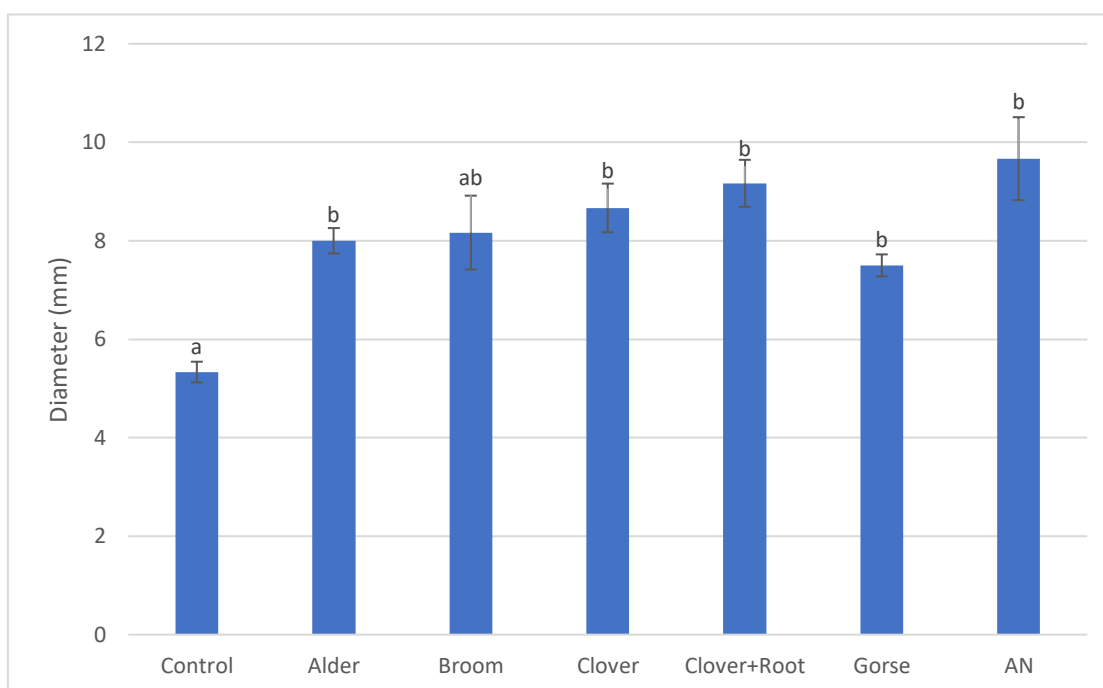
Throughout the experiment, sturdiness of the plants was assessed through a qualitative measure of bendability and noted on a scale 1-5. All plants apart from the control increased in sturdiness during the experiment (Fig. 12). The sturdiness followed a similar pattern to plant height, where AN and clover+root treatments tended to get higher scores. At the final harvest, stem diameter was measured and kale plants from all treatments measured 7.5-9.7mm, and kale from all treatments but broom had significantly larger stem diameter than the control (5.3mm) ( $P < 0.001$ ) (Fig. 13).

Stems had marks left from where leaves had grown, and at the final harvest it was also possible to count how many leaves each plant had produced during its lifetime. Plants grown in broom, clover and clover+root treatments produced significantly more leaves (mean 17.8, 17.2 and 18.3 respectively) throughout the experiment compared with the control (mean 14.3 leaves) ( $P < 0.01$ ) (Fig. 14).

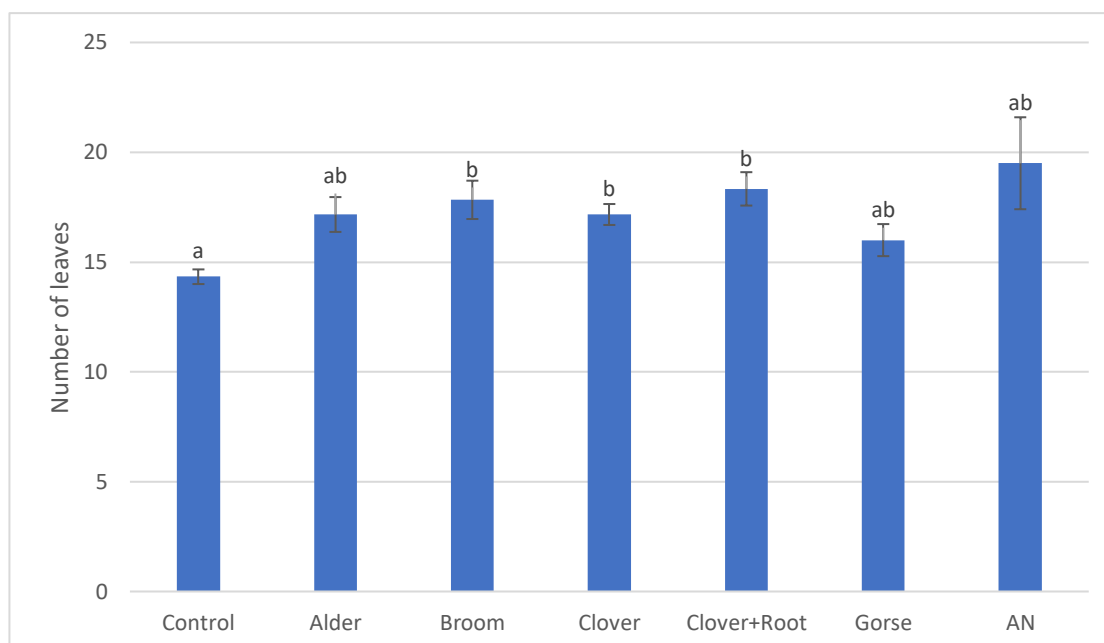


**Figure 12** Sturdiness of plants throughout the experiment rated 1-5 on a qualitative scale of stem bendability. Means  $\pm$  SEM. No statistical test was carried out.





**Figure 13** Diameter of crop stem at harvest 138 days after sowing. Means  $\pm$  SEM ( $n=6$ ), letters indicate statistical significance at  $P<0.05$



**Figure 14** Total number of leaves produced by crop throughout the experiment. Means  $\pm$  SEM ( $n=6$ ), letters indicate statistical significance at  $P<0.05$ .

Whilst all crops were dominated by a green (5G 2/4) leaf colour, the control and gorse treatments had some more purple (7.5P 1/4) leaves than other treatments,

especially at the start of the experiment. After frost, all treatments saw a noticeable increase in bluegreen (2.5 BG 2/4) leaf colour (Fig. 15).

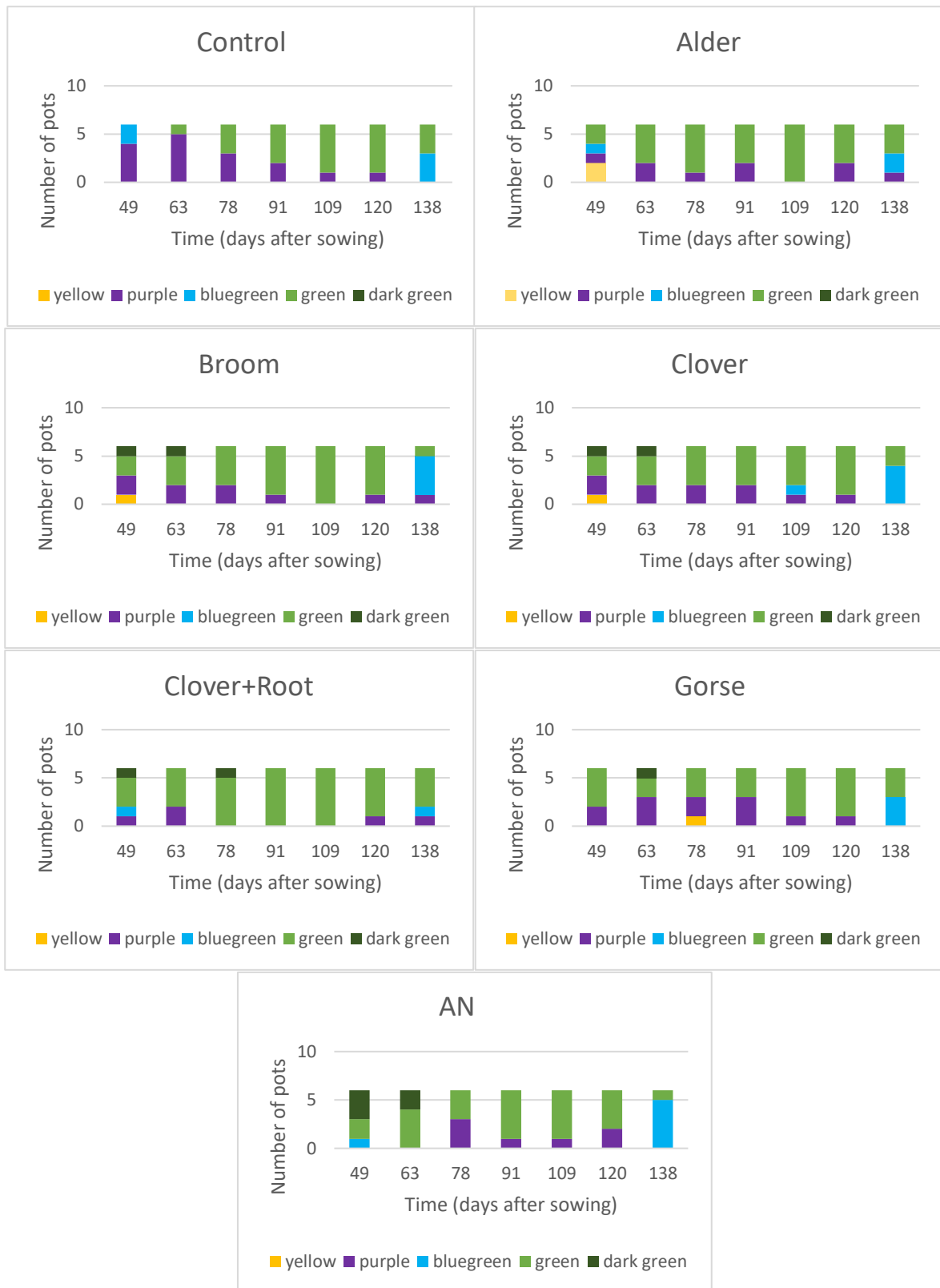
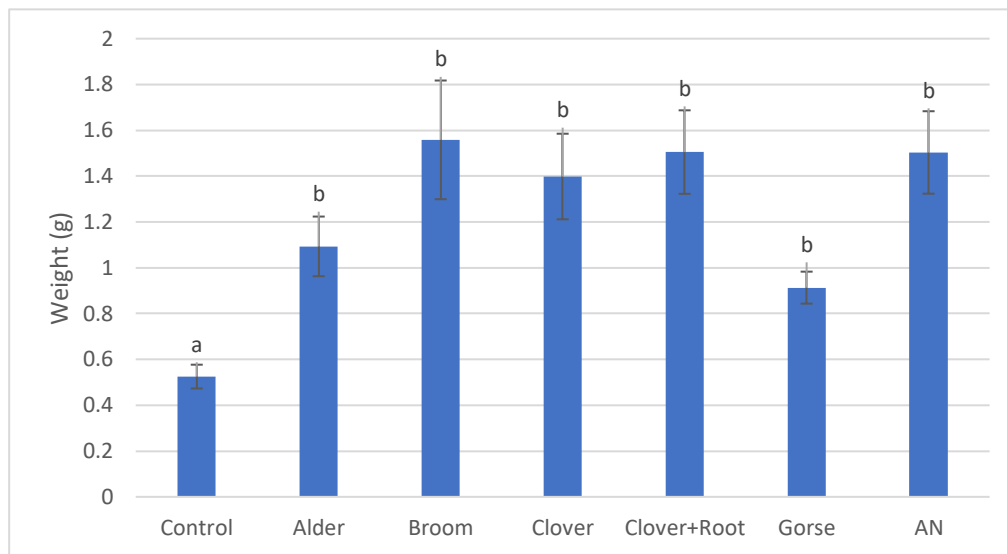


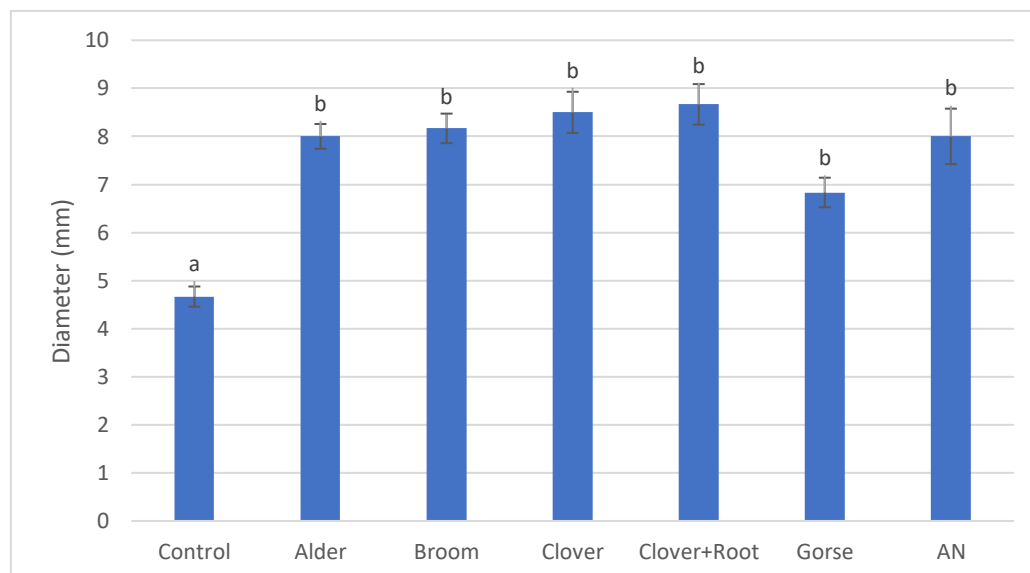
Figure 15 Leaf colour recorded per number of pots of each treatment throughout the experiment.

### 5.3 Root structures of crop

There was no significant difference ( $P>0.05$ ) in weight or length of roots between treatments at the harvests 34 and 82 days after sowing. At 138 days after sowing, kale plants from all treatments had significantly more root mass than the control (0.52g mean dry weight) ( $P<0.01$ ), with the highest root mass recorded in broom (1.55g), clover+root (1.51g) and AN (1.50g) treatments (Fig. 16). Correspondingly, at 138 days after sowing all treatments had a significantly higher root diameter than the control (mean 4.67mm diameter) ( $P<0.001$ ) (Fig. 17). The largest mean diameters were measured in plants with the clover+root (8.67mm), clover (8.5mm) and broom (8.17mm) treatments.

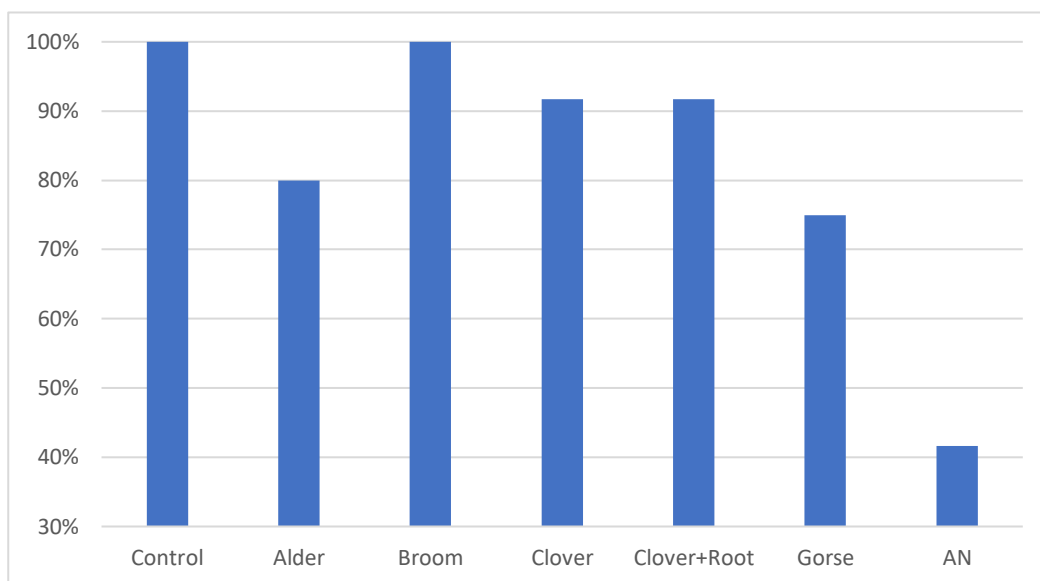


**Figure 16** Root dry weight at harvest 138 days after sowing. Means  $\pm$  SEM ( $n=6$ ), letters indicate statistical significance at  $P<0.05$ .

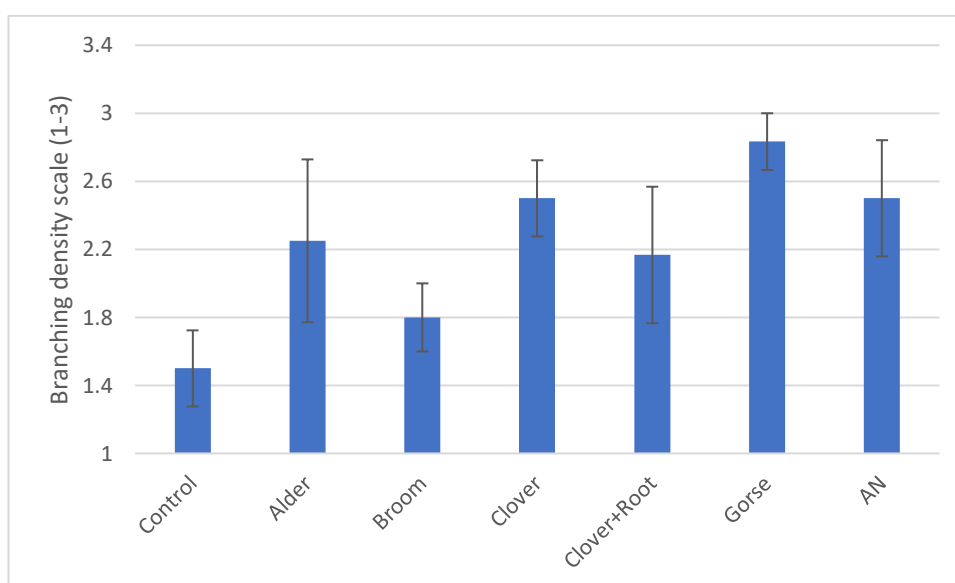


**Figure 17** Root diameter at harvest 138 days after sowing. Means  $\pm$  SEM ( $n=6$ ), letters indicate statistical significance at  $P<0.05$ .

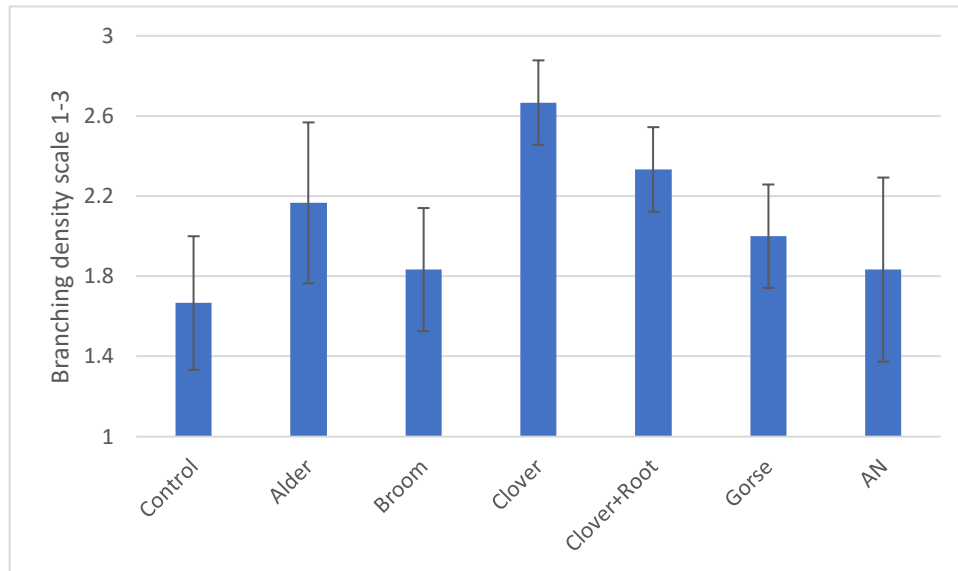
In terms of root morphology, 75-100% of plants in the perennial green manures developed tap roots, whereas the proportion of tap roots in AN was lower at 42% (Fig. 18). Where there was no taproot present, a high-density cluster of lateral roots formed immediately below the soil surface. Root branching density was assessed on a scale of 1-3 (low, medium, high) at the harvests 82 and 138 days after sowing. There was no significant difference ( $P>0.05$ ) in root branching density between treatments at either harvest but the control and broom treatments rated lowest at both occasions (Figs. 19 and 20). There was no significant difference ( $P>0.05$ ) in length of taproot or lateral roots between treatments.



**Figure 18** Percentage of plants that had developed taproots at harvests 82 and 138 days after sowing ( $n=12$ )



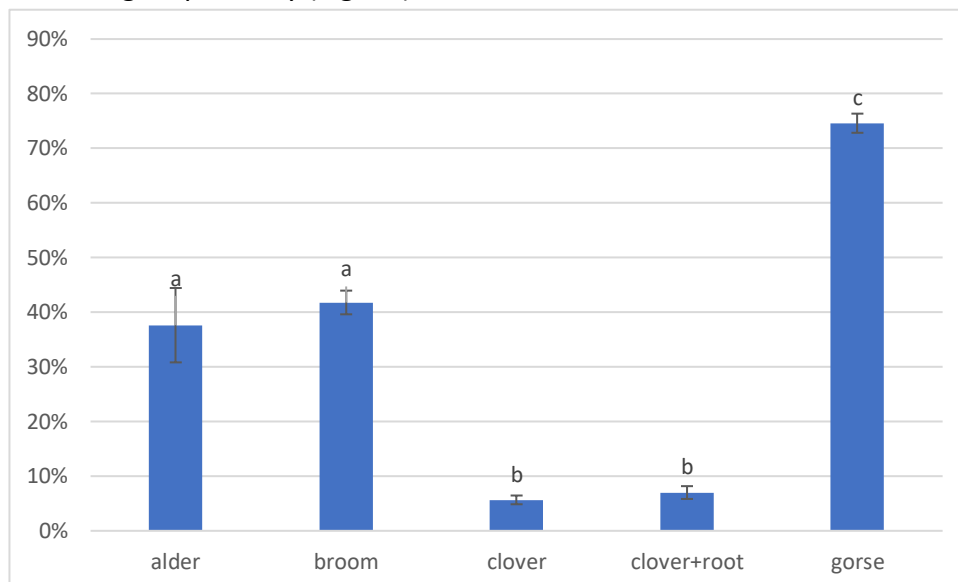
**Figure 19** Root branching density 82 days after sowing, assessed on a qualitative scale of 1-3 (low, medium, high). Means  $\pm$  SEM, no statistical difference at  $P<0.05$ .



**Figure 20** Root branching density 138 days after sowing, assessed on a qualitative scale of 1-3 (low, medium, high). Means  $\pm$  SEM, no statistical difference at  $P < 0.05$ .

#### 5.4 Decomposition rates of PMGMs

There were very highly significant differences ( $P < 0.001$ ) between the decomposition rates of the green manures. After 102 days, gorse had the slowest decomposition rate with an average 75% biomass remaining, alder and broom had 38% and 42% biomass remaining respectively, and clover and clover+root had 6% and 7% biomass remaining respectively (Fig. 21).



**Figure 21** Remaining litter mass in litterbags after 102 days shown in % of original biomass weight. Means  $\pm$  SEM ( $n=5$ ), letters indicate statistical significance at  $P < 0.05$ .

## 6. Discussion

### 6.1 Fertilisation effect of PMGMs

The results indicate that kale crops fertilised by PMGMs achieve similar biomass production rates to those fertilised by conventional green manures and even mineral fertiliser (Fig.10), corroborating results of previous PMGM studies (Ward, 2020; Ward, Chadwick and Hill, 2023). It should be noted that, for the sake of comparison within the experiment, AN fertiliser was applied in one dose at the time of sowing. Farmers are now recommended to use split applications of mineral fertiliser throughout the growing season (AHDB, 2021), and the comparative growth rates seen in the experiment may not be the same in such a situation. Gorse was the only PMGM treatment that had significantly less biomass weight than the AN fertiliser and clover+root treatments at the end of the experiment, indicating a larger initial immobilisation of N and slower decomposition rate. This can be explained by its high C:N ratio (19:1) and can also be due to the presence of secondary metabolites such as phenolic compounds inhibiting decomposition in gorse (Chomel *et al.*, 2016; Pardo-Muras *et al.*, 2020). All PMGM treatments, including gorse, had significantly higher biomass than the control at the end of the experiment, indicating that all treatments had some degree of N release within the 138 days.

Early fast growth of AN fertilised crops suggests a faster availability of N in these pots. By the end of the experiment however, AN treated plants neither had more height or more leaves than other crops, indicating that the fast early growth did not necessarily lead to more harvestable produce in a crop with a long growing season such as kale. Other studies have found that rates of kale growth and harvestable produce were not significantly impacted by the type of organic N fertiliser applied, but rather the rate of N release (Yoder and Davis, 2020).

Clover and clover+root treatments were included in the experiment to measure the effect of a conventional green manure tilled into the soil (with root) and in a cut and carry method (no root included), which has been studied in previous PMGM experiments (Ward, 2020; Ward, Chadwick and Hill, 2023). Whilst there were no significant differences between the treatments, clover+root performed consistently better throughout the experiment, with higher averages of biomass height and weight (Figs. 8-11). Clover+root had a slightly higher C:N ratio of 13:1 compared to clover 12.3:1, and slightly slower decomposition rate. Including the N fixing root nodules in the clover+root treatment may however have provided more N in a plant available form. A significant amount of clover N is located in the root system (Høgh-Jensen *et al.*, 2004) where it is typically at a slightly higher concentration than in the shoot of red clover (Thilakarathna *et al.*, 2017). The rate of release of mineral N from N-fixing nodules has been shown to be correlated to the form of N in the nodule rather than its C:N ratio or organic N content (Wardle and Greenfield, 1991).

Although pots of a substantial size were used, and there was no evidence of root binding at the final harvest of the experiment, the nature of being in a pot will have

affected all the crops (Poorter, Bühler, *et al.*, 2012). None of the plants grew to a size that the cultivar is expected to achieve in the field (Yoder and Davis, 2020).

## 6.2 Pollution effect of PMGMs

As with other organic amendments, using PMGMs entails less GHG emissions than mineral fertilisers due to the lower fossil fuel and energy input required in their production (Lehtinen *et al.*, 2014). The perennial nature of PMGMs further means that the associated soil C loss and detrimental impact on soil health from tilling of annual green manures is avoided (Chen *et al.*, 2020; Cooper *et al.*, 2021).

Although there was no measurement of volatilisation and leaching in this experiment as there was no access to laboratory equipment, it is expected that N loss rates of the PMGMs were lower than the clover and AN treatments. Previous experiments have measured low volatilisation rates of  $\text{NH}_3$  and shown that the concentration of  $\text{NO}_3^-$  in the soil was lower for PMGMs than for clover and AN treatments, indicating a lower leaching risk (Ward, 2020; Ward, Chadwick and Hill, 2023). Mineral fertiliser and conventional clover green manures have been shown to be very prone to leaching when applied at higher rates than the crop uptake capacity. Depending on management techniques, more than half of the N applied can be lost through leaching for both mineral and green manure applications (Bergström and Kirchmann, 2004; Campiglia *et al.*, 2011; Smith *et al.*, 2018; Mahmud *et al.*, 2021).

Lower  $\text{NO}_3^-$  concentrations in the soil could however indicate a lower N availability to plants (Whalen, 2014; Hirsch and Mauchline, 2015), but this is difficult to determine as it may also be due to synchronised N release to plant uptake (Crews and Peoples, 2005; Jackson, Burger and Cavagnaro, 2008; Chen *et al.*, 2014). As there was no significant difference in growth rates of alder, broom, clover and AN treated plants, it is expected that the lower  $\text{NO}_3^-$  were possibly only growth limiting in the gorse treatment, as it was the only PMGM treatment to have significantly less biomass growth compared to the AN treatment.

The expected low  $\text{NO}_3^-$  levels in the soil also have an impact on the  $\text{N}_2\text{O}$  emissions, where lower  $\text{NO}_3^-$  availability reduces the  $\text{N}_2\text{O}$  produced during denitrification (Charles *et al.*, 2017). PMGMs have been shown to have  $\text{N}_2\text{O}$  loss of 0.3-1.2% of the N applied, compared to 5.3% from clover (Ward, 2020). However, the  $\text{N}_2\text{O}$  loss rates may increase in subsequent seasons as the C-rich PMGMs continue to decompose over a longer timeframe (Pugesgaard *et al.*, 2017), but this has not yet been examined in PMGM experiments.

Using PMGMs may thus be an integral part of reducing leaching rates and  $\text{N}_2\text{O}$  emissions in organic agriculture.

## 6.3 Germination rates

There was no significant effect of PMGMs on germination rates of crop or weed seeds (Figs. 6-7), indicating that the presence of phenolic compounds in gorse and broom did not have an impact on seed germination as reported by others (Pardo-

Muras *et al.*, 2018, 2020; Pardo-Muras, Puig and Pedrol, 2019, 2022). Pardo-Muras *et al.* (2020) recorded germination 30 days after incorporation of gorse and broom slashed to 1cm pieces in a pot experiment in a green house. Larger surface areas of smaller green manure pieces, higher average temperatures and higher watering rates may have contributed to a faster decomposition of the green manures in their experiment (Krishna and Mohan, 2017) and consequently recorded effects of phenolic compounds earlier. As measured decomposition rates were low for gorse and broom, it is possible that the allelopathic effects of phenolic compounds would be noticeable first at later stages or for subsequent crops. Additionally, it may be possible that varieties of weeds that self-germinated were not sensitive to the phenolic compounds present, as some seem less affected than others (Pardo-Muras *et al.*, 2020) and can be dependent on seed size (Kruidhof *et al.*, 2011).

Germination rates were slightly lower in the AN treatments than any other treatment, although not statistically significantly so. It is likely that the initial high concentrations of  $\text{NH}_4^+$  of the AN treatments were slightly toxic and responsible for inhibiting germination (Pan *et al.*, 2016), although further signs of toxicity such as discoloration of leaves or roots or rotting roots were not recorded (Britto and Kronzucker, 2002). The lower germination rates in the AN treatment may be of limited relevance as it is no longer common or recommended to apply N mineral fertiliser in such a large dose at one time at the time of sowing (AHDB, 2021), but was only done so in the experiment to enable comparison between treatments.

#### 6.4 Root structures of crop

The noticeable difference in taproot morphology between AN and green manure treated plants (Fig. 18) can be explained by the nature of the nutrient availability. The AN treated plants tended to have shallow root systems with a higher density of thinner lateral roots without tap roots, which is consistent with high nutrient availability in the soil surface (Freschet *et al.*, 2021). High nutrient availability can however also lead to thicker roots, which was evident in the experiment as the root biomass was significantly higher in all treated pots compared to the control. The distribution of nutrients in the soil impact the root structure. Increasing nutrient limitation in the soil has been shown to increase the specific root length (SRL) (Holdaway *et al.*, 2011), i.e. the length of root per unit of weight, with higher SRL meaning thinner roots (Freschet *et al.*, 2021). This is because the energy investment in root growth for further exploration is rewarded by further access to nutrients through greater resource acquisition capacity (Zangaro *et al.*, 2008; Freschet *et al.*, 2018). Other studies have however reported decreased SRL with decreased nutrient availability (Freschet *et al.*, 2018), the reason for which is increased root density rather than diameter to ensure root longevity in a low nutrient environment (Miller and Cramer, 2005; Eissenstat *et al.*, 2015). Lower root branching density and longer root hairs have also been recorded at lower N supply (Freschet *et al.*, 2018), increasing the volume of soil that could be explored for resource acquisition, which is consistent with the observation in this experiment that the control had consistently lower root branching density than the treated pots (Figs. 19-20).



Especially in coarse soils, plants with longer and thinner roots have an advantage in terms of long term nutrient acquisition (Holdaway *et al.*, 2011), which may mean that PMGM treated plants have an advantage over AN treated plants in the long term, with root systems developing earlier on to cover a large volume of soil. Additionally, the lack of tap root development in a majority of the AN treated plants reduces their resilience and makes them more susceptible to incur damage in extreme weather such as high winds, heavy rainfall or drought (Lynch, 2022; Kalra, Goel and Elias, 2023).

### 6.5 Decomposition rates of PMGMs

The decomposition rates measured (Fig. 21) followed a general trend predicted by C:N ratios, where low C:N ratios decomposed significantly faster than high ones (Krishna and Mohan, 2017). Clover had the lowest C:N ratio of 12.3:1, and clover+root a slightly higher at 13:1 – although this did not significantly impact the decomposition rate. These C:N ratios are comparable to others measured in the literature, ranging from 10.1:1 – 17.9:1 (Thilakarathna *et al.*, 2017; Ward, 2020), the difference possibly being due to the age of the clover plants.

Gorse and broom treatments had 1-2% higher N content than has been measured in Pardo-Muras *et al.* (2020), and gorse had a lower C:N ratio than measured by Ward (2020) (19:1 compared to 26.8:1), probably due to the high flower content of the treatments. Alder had the same 15.1:1 C:N ratio to what has been seen in other studies (Ward, 2020).

The growth rates of the kale broadly mirrored the decomposition rates, where clover and clover+root treatments saw slightly higher plant height and biomass earlier on in the experiment. By the end of the experiment, there was no significant difference between the clover and alder and broom treatments, indicating that the decomposition of alder and broom had led to N release.

It should be noted that the full percentage of biomass that had left the mesh bag may not have been broken down in the soil and become accessible to microbial digestion and subsequent plant uptake. This may be especially true of the gorse treatment, where phenolic compounds contribute to recalcitrant polymers remaining undigested in the soil for some time (Pardo-Muras *et al.*, 2020). The decomposition rate is thus merely indicative of the rate at which nutrients may become available to plants. The rate of decomposition may also change over time if there is consistent use of one treatment, when the microbial population adapts to the specific nature of the addition – the so called home field advantage (Ayres *et al.*, 2009).

### 6.6 Timing of N release

The high leaching rates of both mineral fertilisers and conventional green manures tend to be caused by too much N being mineralised and in plant available form

before there is sufficient plant demand (Crews and Peoples, 2005). The slower decomposition rates of PMGMs creates a slower and longer release of N, meaning that for a targeted N application, good knowledge of decomposition rates and N release is needed and the PMGMs may need to be applied early in the season, or over several seasons to release N sufficient for plant demand (Ward, Chadwick and Hill, 2023).

Additionally, adding woody perennial green manures increases the fraction of slowly decomposing plant matter in the soil, contributing to stabilising organic matter and building up soil function, as well as being a pool of N that will be released slowly to subsequent crops (Crews and Peoples, 2005; Whalen, 2014). Consequently, it is important to note that the N used by crops in green manure systems does not always come directly from the decomposing organic matter – which means that studies measuring N uptake efficiency in one season, e.g. through labelled  $^{15}\text{N}$  in legumes and green manures, tend to underestimate the amount of N supplied (Trinsoutrot *et al.*, 2000; Crews and Peoples, 2005). Instead, as the N is added in combination with large amounts of C, the N is immobilised in microbial biomass, and older immobilised microbial N is released, mineralised and made available for plant uptake (Murphy, Fillery and Sparling, 1998; Trinsoutrot *et al.*, 2000).

Using PMGMs could shift the focus from supplying the exact right quantity of N at the right time to match N demand, as is advocated in precision agriculture, to building up the capacity of the soil to store and release N when needed for crop growth. It follows that in order to assess the viability and efficiency of N supply through PMGMs, experiments need to be conducted over several growing seasons.

## 6.7 Implications of PMGM use on the availability of organic N

In agricultural science, the dynamics and impacts of organic N from mineral or organic additions have not been studied to the same extent as the inorganic N, leading to a dominant narrative that plants exclusively take up N in the mineral forms of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Farzadfar, Knight and Congreves, 2021). Organic N in the soil comes from the addition of organic matter such as manures, crop residues, and dead animal and microbial matter, and is most commonly found in the form of poly-amino compounds such as proteins, amino acids, oligomers and polymers (Schulten and Schnitzer, 1997; Roberts and Jones, 2008; Hu *et al.*, 2020).

Crop uptake of organic N is attracting increasing attention and recent studies have confirmed that crops take up organic N in the forms of smaller compounds such as amino acids and peptides, as well as larger polymers such as proteins (Hill *et al.*, 2011; Warren, 2014; Moreau *et al.*, 2019). Compared to  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , taking up organic N requires less energy from the plant as it can occur by passive diffusion and as the N is already in a compound form and does not need added C to build, for instance, proteins, the so called carbon bonus (Franklin *et al.*, 2017; Moreau *et al.*, 2019; Farzadfar, Knight and Congreves, 2021). The significance of organic N uptake in crops is however still debated, as concentrations of plant available organic N sources in the soil can be very low (Roberts and Jones, 2008; Jan *et al.*, 2009; Warren, 2017).

Others have suggested that it is the rate of replenishment, rather than the concentration, of e.g. amino acids in the soil solution that determines the significance for plant nutrition (Moreau *et al.*, 2019). Amino acids are consistently at a low concentration across ecosystems, but are replenished over 1000 times a day, which is considerably faster than the rates of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  production in the soil (Andresen *et al.*, 2015; Moreau *et al.*, 2019). In conditions of low inorganic N availability, crops have been shown to take up a larger fraction of organic N (Gioseffi, De Neergaard and Schjoerring, 2012; Czaban *et al.*, 2016; Dion *et al.*, 2018; Czaban and Rasmussen, 2021). Some laboratory studies have seen higher crop root:shoot ratios and N efficiency levels when taking up organic N rather than mineral N, but this has not been demonstrated in field trials (Franklin *et al.*, 2017).

The use of PMGMs increases the availability of organic N in the soil compared to mineral fertilisers as the organic material breaks down in situ. Due to the higher C:N ratios and recalcitrant compounds, the release of plant available organic N from PMGMs and decomposing microorganisms would occur over a longer timescale than for traditional green manures such as clover. This could have a larger effect on crop nutrition than predicted by the PMGM decomposition and mineralisation rates, as organic N has been shown to remain in the soil N pool and N derived from decomposing organic materials may be taken up by crops over several seasons following application (Dessureault-Rompré *et al.*, 2013; Sebiló *et al.*, 2013; Pugesgaard *et al.*, 2017; Pullens *et al.*, 2021). There is evidence to suggest that organic N fluxes in the soil are more synchronised with crop root uptake capacity than inorganic N fluxes (Brackin *et al.*, 2015). Additionally, as organic N uptake does not involve nitrification and denitrification processes, the risk of losing N to leaching and as  $\text{N}_2\text{O}$  emissions is reduced, which could contribute to increased overall agricultural NUE (Ward, 2020).

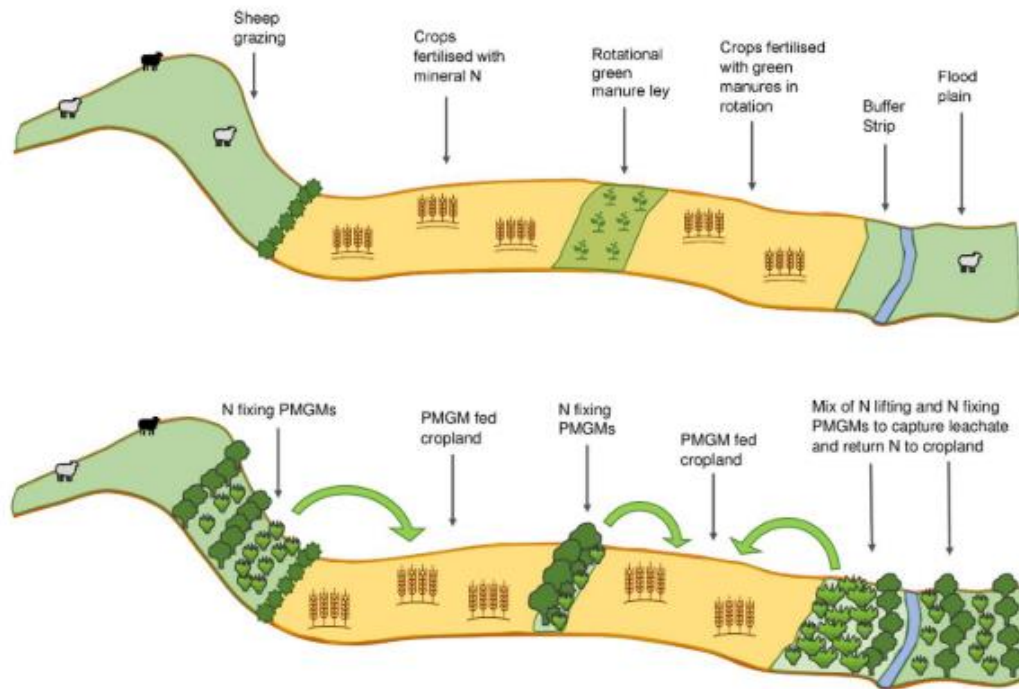
## 6.8 Choice of cultivars

Modern kale cultivars are bred to higher N availability conditions and thus may be “heavy feeders”. Yoder and Davis (2020) reported that an F1 hybrid kale cultivar had lower leaf N sufficiency than other kale cultivars with the same organic N fertiliser treatment. Additionally, modern cultivars are selected for high inorganic N availability, which may have led to a decreased capacity to take up organic N (Hirel *et al.*, 2011). The use of PMGMs may require the careful selection of cultivar, a return to landrace varieties which are expected to be more efficient at taking up organic N, or specifically including organic N uptake traits in breeding of cultivars (Reeve *et al.*, 2009; Soumare *et al.*, 2020; Farzadfar, Knight and Congreves, 2021).

## 6.9 Land use efficiency

PMGMs such as alder, gorse and Scotch broom can grow on flooded, steep, depleted or otherwise marginal soils (Paynter *et al.*, 2000; Claessens *et al.*, 2010; Atlan *et al.*, 2015), thus reducing the need for agriculturally productive land to be set aside to biologically fix N (Ward, Chadwick and Hill, 2023) (Fig. 22). Ward, Chadwick and Hill (2023) further suggest that the perennial nature of PMGMs will support other ecosystem functions such as carbon sequestration and biodiversity important for agricultural system (Balmford *et al.*, 2018; Pilling, Bélanger and Hoffmann, 2020).

Incorporating stands of perennial plants between arable areas can also intercept leached N and provide shelter to reduce erosion from wind and rain (Bowles *et al.*, 2018; Diederich *et al.*, 2019; Caulfield *et al.*, 2020) contributing to the overall NUE of the system.



**Figure 22** Present (above) and future PMGM (below) agricultural landscape scenarios (Ward, Chadwick and Hill, 2023, p.56). PMGMs are grown in bio-service areas on marginal land and used for N fertilisation instead of mineral fertilisers. The amount of land for arable crops is equal in both scenarios.

## 7. Conclusion

The current agricultural system requires urgent transformation as it is a major contributor to climate change which, beyond its high energy and fossil fuel use, transgresses the boundaries for a safe and inhabitable planet for humans in terms of biosphere integrity, land-system change, freshwater use and nitrogen (N) flows (Steffen *et al.*, 2015; Rockström *et al.*, 2017; Gerten *et al.*, 2020; Chang *et al.*, 2021). The plant-available reactive nitrogen essential for crop growth is predominantly produced through the fossil fuel intensive Haber-Bosch process (Fageria and Baligar, 2005; Hirsch and Mauchline, 2015; Abdalla *et al.*, 2019; Soumare *et al.*, 2020). Application of the resulting mineral fertiliser is inefficient and more than 50% of the N applied is lost to the environment where it causes ecosystem damage and greenhouse gas emissions (Cameron, Di and Moir, 2013; Batty, Aneja and Schlesinger, 2017; Houlton *et al.*, 2019; Carr *et al.*, 2020). Organic alternatives using biological N fixation, such as green manures, do not have the associated high energy and emissions cost in production, but require arable land to be set aside for their growth and can, depending on timing and quantity of application, incur as high leaching rates as mineral fertilisers (Crews and Peoples, 2005; Smith *et al.*, 2018; Carr *et al.*, 2020).

PMGMs were explored as an alternative to conventional green manures, where their perennial nature and capacity to grow on marginal land means that arable land does not need to be set aside for their growth. Using PMGMs instead of Haber-Bosch produced mineral fertiliser has the potential to reduce the fossil fuel reliance and GHG emissions of the agricultural system.

In terms of N supply, this study has shown PMGMs to be a viable alternative to traditional green manures, and can yield kale crop growth levels comparable to AN mineral fertiliser in a pot experiment. Although AN treated kale plants saw fast early growth, they did not have significantly more biomass at the end of the growing season than kale plants treated with alder, broom or the conventional green manure red clover. Gorse was the only PMGM that had significantly lower biomass than AN at the end of the experiment, indicating slower decomposition and a higher N immobilisation rate than the other PMGMs. PMGM decomposition rates followed their C:N ratio, where higher C:N ratio species, such as gorse, decomposed slower than alder and broom. The slower decomposition is expected to mean lower volatilisation and leaching rates of PMGMs compared to conventional green manures.

Achieving synchrony between the release of N and the crop N demand is a challenge in traditional green manures, that are commonly tilled into the soil and subsequently release N quickly and before there is adequate crop uptake capacity, which can lead to substantial N losses through leaching (Bergström and Kirchmann, 2004; Crews and Peoples, 2005; Carr *et al.*, 2020). The necessary cut and carry method of PMGMs means that the timing of application can be adjusted to achieve N supply and demand synchrony. However, due to the woody nature and high C:N ratios of the PMGMs, decomposition is considerably slower than traditional green manures and

may take place over several growing seasons. PMGMs may thus be better suited for crops with a longer growing season.

The application of PMGMs may also contribute to a build-up of a latent organic N pool in the soil. There is still no conclusive evidence about the extent of crop capacity to take up organic N, but studies indicate that it has potential as a crop N source, has a carbon advantage to the plants and can further lower pollution effects as nitrification and denitrification processes are bypassed (Czaban and Rasmussen, 2021; Farzadfar, Knight and Congreves, 2021). Modern cultivars may however be bred to grow well in high mineral N conditions, and using PMGMs may necessitate using landrace cultivars or breeding for organic N uptake.

There was no indication that allelopathic compounds in any of the PMGMs inhibited germination of kale seeds. PMGM and clover additions contributed to the development of tap roots and larger root structures of the kale crop than the AN treated plants, which could mean higher climate resilience and capacity to withstand unpredictable and changeable weather patterns in the crops with organic amendments.

As PMGMs can be grown on marginal land, arable land does not need to be set aside for biological N fixation. This increases the overall productive land area whilst avoiding the emissions and energy consumption associated with mineral fertiliser production. Additional benefits are projected in terms of perennial bio-service areas of PMGMs contributing to increased C sequestration, increased biodiversity and wind and erosion protection.

## 7.1 Limitations

Due to the limited time frame of the research project, an annual crop had to be grown and thus the longer-term effects of PMGM use could not be studied or accounted for. The slow decomposition rates of PMGMs meant that the release of N was only just commencing towards the end of the experiment, and any further growth effects of the N release were thus not recorded.

Rudimentary measuring equipment and a very low budget for the experiment meant that some measurements pertaining to crop roots, sturdiness and colour were not done in as much detail or as systematically as would be appropriate. Additionally, it was not possible to monitor N release, volatilisation and leaching. A continuous monitoring of the N levels in the soil and kale crop would have given clearer indications of the N release and uptakes rates, and given better understanding of the organic and mineral N dynamics. Limited storage capacity and experimental set up area also meant that processing and storage of treatments and soil may have been substandard and may somewhat reduce the applicability of the laboratory analysis of the treatment and soil properties.

Undertaking a pot experiment meant that the crops were not subjected to the complex dynamics of field conditions, limiting the applicability of the results somewhat, and the crops may have been adversely affected by the constrictions of

the pots (Scheiner and Gurevitch, 2001; Kawaletz *et al.*, 2014), although this was not noticeable at the time of harvest.

Processing and application methods of PMGMs were beyond the scope of this study, and thus no consideration has been given to whether fresh, dried, composted, mulched or dug in PMGM materials have differing effects on crop growth.

## 7.2 Implications

This study corroborates early studies into PMGM use, showing that PMGMs can achieve similar growth rates to conventional green manures and even mineral fertilisers (Ward, 2020; Ward, Chadwick and Hill, 2023). The added features that PMGMs grow on marginal land and do not require arable land to be set aside for their growth has the potential to improve NUE and land use efficiency in organic agriculture. There is further multifunctionality in PMGMs as they can increase biodiversity and ecosystem function, act as buffer strips to prevent leaching, and increase climate resilience through wind and flooding protection, with implications for the current research into sustainable agriculture and debates about the viability of relying on biological N fixation (Soumare *et al.*, 2020).

If PMGMs can be applied on a large scale with a comparable result to synthetically produced mineral nitrogen fertiliser, their use can address the concerns of transitioning the nitrogen fertiliser production to net-zero emissions, where current projections indicate an increase in land, water and energy use (Rosa and Gabrielli, 2023).

This study indicates that there is an important research need for studying the organic N fraction of the soil and its effect on crop nutrition. Shifting crop nutrition focus from exclusively considering mineral N dynamics to including organic N will have implications for how N amendments are treated in the future, and may enable more long-term nutrient management plans where soil function is built up through added organic matter, contributing to more sustainable food production.

This research also has implications for the conventions within invasive species management, where practices for dealing with gorse and broom, for example, have been developed without consideration for their potential as biological fertilisers. Continuous harvest and use of them would have the combined benefits of limiting their expansive growth, gaining N fertiliser additions, and avoiding buying in mineral fertilisers with high energy, emissions and financial costs.

## 7.3 Further research

Further research of PMGMs in field trials and different management practices is needed. This includes trialling and building a database of different PMGM species, as well as comparing the use of fresh and processed, e.g. dried, composted or anaerobically digested, PMGMs. Current agricultural practices and machinery are adapted to mineral fertilisers or conventional green manures, posing practical challenges of how to harvest, process, store and apply PMGMs, which merit further investigation and experimentation.

To be able to account for the long-term effect of slow decomposition and latent organic N build up in the soil, PMGM experiments taking place over several growing seasons are needed. Longer experiments would also be able to further investigate the importance of application timing to achieve N supply or demand synchrony, and to what extent a build-up of organic N in the soil influences the necessity for precision in timing applications. It would also enable the monitoring of volatilisation, leaching and N<sub>2</sub>O emissions as the PMGMs break down over time.

To date, PMGM experiments have focussed on N dynamics. Further research is needed into the dynamics of other nutrients, especially phosphorus (P) and potassium (K), as they are also essential to crop growth.

The allelopathic effects of gorse and broom inhibiting weed germination reported elsewhere in greenhouse experiments (Pardo-Muras, Puig and Pedrol, 2019; Pardo-Muras *et al.*, 2020) were not noticed in this study. Further outdoor and field experiments are required to ascertain their potential allelopathic effects in field conditions.

The research area of organic N for crop nutrition is growing quickly. The fate of organic N when using PMGM treatments merits further investigation, especially to what extent crops take up organic N compounds originating from PMGMs. This may shift the focus from mineralisation rates to a broader understanding of nutrient dynamics and contribute to a more holistic understanding of the role of organic amendments in agriculture.



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