Is Green Manure from Riparian Buffer Strip Species an Effective Nutrient Source for Crops?

JOURNAL OF ENVIRONMENTAL QUALITY

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Journal:	*Journal of Environmental Quality*
Manuscript ID	JEQ-2017-11-0422-RM.R2
Manuscript Type:	Special Section: Riparian Management
Technical Report Subtypes:	Plant and Environment Interaction
Date Submitted by the Author:	n/a
Complete List of Authors:	Brown, Lawrie; James Hutton Institute Kazas, Charalampos; University of Edinburgh School of GeoSciences Stockan, Jenni Hawes, Cathy; James Hutton Institute Stutter, Marc Ryan, Casey; University of Edinburgh School of GeoSciences Squire, Geoff; James Hutton Institute George, Timothy; James Hutton Institute
Keywords:	Agricultural sustainability, green manure, nutrient recycling, riparian buffers

Is green manure from riparian buffer strip species an effective nutrient source for crops?~~Can biomass from riparian buffer strip species be used as an effective source of green manure for crops~~?

[1]Lawrie K Brown, [2]Charalampos Kazas, [1]Jenni Stockan, [1]Cathy Hawes, [1]Marc Stutter, [2]Casey M Ryan, [1]Geoffrey R Squire, [1*]Timothy S George

The James Hutton Institute, Aberdeen AB15 8QH and Dundee DD2 5DA, Scotland, UK

[2]The School of Geosciences, University of Edinburgh, Crew Building, The King's Buildings, Alexander Crum Brown Road, Edinburgh EH9 3FF, UK

[*]Corresponding author: tim.george@hutton.ac.uk

Abbreviations

acc, accumulation
ANOVA, analysis of variance
C, carbon
con, concentration
DW, dry weight
ghg, greenhouse gas emissions
GM, green manure
INM, integrated nutrient management
K, potassium
LSD, least significant difference
N, nitrogen
NH_4, ammonium
NO_3, nitrate
P, phosphorus
PO_4, phosphate
TOC, total organic carbon

Core Ideas

- We need to 'close the loop' on nutrients that become environmental pollutants
- Green manure from riparian buffer strips cannot replace chemical fertilisers
- Buffer strip green manure integrated with fertiliser promotes plant growth
- Common riparian buffer species have variation in nutrient accumulation
- Selection of~~ed single species green manure~~ P and N accumulating plants~~s~~ do not provide a superior ~~nutrient source~~green manure

Abstract

~~The sustainability of agriculture needs to be improved. We~~Agriculture needs to reduce the inputs of inorganic fertilisers and 'close the loop' on nutrients that can otherwise become environmental pollutants. ~~We~~ This can be achieved ~~this~~ by promoting recycling of nutrients within the agricultural landscape. We investigated the extent to which plants found in riparian buffer zones, have potential to provide nutrients to crops as a green manure, through plant growth and decomposition studies. Under controlled conditions, s~~S~~pecies typical of Scottish riparian buffer strips were tested for their ability to accumulate biomass and nutrients in tissue, under N and P replete conditions and whether this ability enhanced the utility of the resulting green manure in promoting crop growth. In this proof of concept study, w~~W~~e found that green manure derived from riparian buffer strips did not effectively replace inorganic fertiliser and only had a significant positive effect on growth, yield and nutrient accumulation in barley when it was integrated with the addition of inorganic fertilisers. The individual species tested varied in the amount of P they accumulated in their tissue (1.38 to 52.73 mg P plant^{-1} ~~38-fold difference~~), ~~and different species had a range of impacts on the availability of C, N and P in soils,~~ but individual species did not differ in their ability to promote yield when used as a green manure. Our results indicate that selecting certain species in the buffer strip, based on their nutrient accumulating abilities, is not an effective way to increase the utility of buffer strip green manure as a nutrient source for crops. ~~Here we demonstrate that the selection of buffer strip vegetation as an effective green manure cannot be done solely by selecting specific species based on their nutrient accumulation characteristics and a number of other considerations should be made to make this technology effective.~~

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Introduction

With increasing demand for food, diminishing resources, unsustainable agronomic practices and environmental change, there is an urgent need to change agricultural production to deliver long-term sustainable food security (Lynch et al., 2007; Gregory and George, 2011). This must be achieved using reduced-input systems in which loss of nutrients from agricultural land to the rest of the environment is reduced to counter the impact on environmental quality.

The accumulation and subsequent mobilisation of large concentrations of nitrogen (N) and phosphate (PO_4) in agricultural soils is a major contributor to surface water pollution and an indicator of imbalance in agricultural systems world-wide (Condron et al., 2013). This is particularly true in Europe, the United States, and Asia where agricultural intensification and profligate use of manures and chemical fertilisers has increased soil nutrient stocks beyond crop requirement (Rosemarin and Ekane, 2016). As the mismanagement of soil continues to push the global boundaries of nutrient sustainability (Steffen et al., 2015), new approaches are needed to more effectively manage crop nutrition.

Nutrient utilisation in agriculture could be improved by recycling nutrients within the agricultural landscape. The transfer of nutrient rich organic materials from zones where nutrients accumulate to zones where nutrients are required for production could contribute to such a solution (Jama et al., 2000; Mankin et al. 2007). This represents a process of 'closing the loop' of nutrient use. The use of green manure, in which plant biomass is produced specifically to provide an input of nutrients to crops, could offset some of the required fertiliser input. This is a relatively common practice in low-input systems in the tropics (Sharma and Mittra, 1988; Becker et al., 1995; Dreschel et al., 1996; Fischler et al., 1999; Gachengo et al., 1999; Jama et al., 2000; Cobo et al., 2002a) and in organic systems in temperate regions (Baggs et al., 2000, Olesen et al., 2007, 2009), but is rare in temperate intensive agricultural systems.

Green manures are grown and used with the intention of improving soil fertility by increasing soil organic matter content and the amount of plant-available nutrients, notably N, P and potassium (K) (Diekman et al., 1993; Peoples et al., 1995; Baggs et al., 2000). Green manure amendments stimulate soil microbial growth and activity, with subsequent mineralisation of plant nutrients (Lundquist et al., 1999; Randhawa et al., 2005; Eriksen, 2005), but the ability to deliver nutrients

depends on the composition of the amendments, timing of application and the prevalent environmental conditions. Plant species for use as green manures must accumulate large amounts of nutrients in their biomass, and these nutrients must be readily released in plant-available forms when the biomass is applied (George et al., 2001). Since availability of carbon (C) substrates largely controls microbial growth in soil, the quality of the green manure material is a key factor governing nutrient release from these materials upon addition to soil (Elfstrand et al., 2007). While the release of N is strongly determined by the C:N ratio in the green manure (the smaller the ratio the greater the release), the release of P is additionally governed by the direct mineralisation of organic P by microorganisms (McGill and Cole, 1981). The addition of green manures also have a number of other benefits associated with the addition of C to the system including reduced erodibility, increased water retention in soils and improved soil structure (Cobo et al., 2002a; Fischler et al., 1999; Zhang and Fang, 2007). Importantly, gGreen manures have ~~also~~ been shown to reduce greenhouse gas emissions (ghg) (Aulakh et al., 2001), but not when integrated with mineral fertilisers (Sarkodie-Ado et al., 2003). However, integrated nutrient management (green manure in combination with mineral fertiliser) is a potentially desirable practice as it can produce more consistent and larger benefits to yield than green manure alone (Sharma and Mittra, 1988; Jama et al., 2000).

To be useful as a green manure, species selected for the purpose must produce large amounts of nutrient rich biomass (~~up to 20 t ha^{-1} of application area;~~ Jama et al., 2000), therefore, it is important that species used should have strategies to maximise nutrient acquisition and accumulation in the above ground harvestable tissue. ~~which~~ These strategies will include root architectural and anatomical changes and alterations to the plants rhizosphere biochemistry through exudation (Brown et al., 2013). Selection for plants with such traits in zones where green manures are being grown should be beneficial.

Riparian buffer strips are landscape features which separate agricultural land from water courses. The vegetation growing in these buffers accumulate nutrients from adjacent fields (Stutter et al., 2009; Stutter and Richards, 2012., Mankin et al., 2007) and as such are potential prime sources of green manure in the agricultural landscape. In the case of riparian buffer zones one of the advantages of using biomass produced therein as a green manure is the removal of nutrients from this accumulation zone. Riparian buffers act as zones of interception of soil, runoff and throughflow from agricultural

fields which may have a high load of inorganic nutrients such as N and P, as well as other agrochemical pollutants (Borin et al., 2005). They have many additional benefits, including promoting biodiversity, sequestering carbon, reducing erosion and providing space for natural fluvial processes (Stutter et al., 2012). By selecting the type and make-up of the vegetation at the initiation of the buffer strips it is possible to provide additional ~~multiple~~ benefits from these landscape features (Schultz et al., 1995) ~~and~~ in the form of valuable material for use elsewhere in the system or in the landscape.

In this paper we investigate ~~some aspects of~~ the ability of green manure produced by harvesting riparian buffer biomass to provide nutrients to~~-~~ spring barley~~crop plants. We investigate~~ and assess whether the utility of such green manure could be enhanced by selecting specific species for growth in riparian buffer zones. In this proof of concept study ~~We~~ our objectives are ~~aim~~ 1) to assess the extent to which ~~harvested~~ biomass harvested from buffer strips can be used as a fertiliser to ~~crop plants~~promote yield and nutrition of spring barley in a typical arable soil of the region, and 2) to quantify the abilities of different plant species, typical of riparian buffers, to sequester N and P in their tissue and then supply these nutrients ~~-~~to ~~crops~~ spring barley when added to the soil as a green manure.

Materials and Methods

Comparison of the effectiveness of green manure from buffer strips (Experiment 1)

Green manure preparation

In an initial experiment, plant material used as green manure, was collected from two different riparian buffer strip sites in Scotland. The first was Balruddery farm (56°29'10'', 3°8'8'', elevation 70-124 m), a 170 ha arable farm near the James Hutton Institute, 10 km west of Dundee, Scotland. The second was from the Tarland catchment in Aberdeenshire in northeast Scotland (centred on 57°53′ N, 2°00′ W, elevation range 145-298 m). Plants from Tarland were harvested during the second week of July 2013 from three sites and stored at -20 °C until the experiment was set up in December 2013. Plants from Balruddery were harvested in December 2013. The average nutrient concentrations in bufferstrip green manure material were 2.6, 12.7 and 183.3 mg kg^{-1} dry weight for P, N and C, respectively, and these were used to calculate biomass addition rates later. The species composition of the different buffer strips can be seen in supplemental Table S1. There were >70 species present at both sites,

and of the dominant species, 8 were common to both. However >20 of the dominant species were unique for either Tarland or Balruddery (Supplemental Table S1), demonstrating key differences in the species composition of the green manures used.

Soil Preparation

Topsoil (0-10 cm depth) of a typical arable soil of the region, characterised as sandy silt loam, Balrownie series, Cambisol (FAO, 1994), with an organic matter content 4.5%, CEC 12 meq 100 g^{-1} and pH 6.0, was collected from a field close to the buffer strips at Balruddery farm. This soil was air-dried and sieved and then ~~was~~ either left non-fertilised (Control), or amended with a range of sources of chopped green manure, combinations of the green manure and chemical fertiliser (integrated nutrient management [INM]) or chemical fertiliser alone, detailed in supplemental Table S2. All treatments were replicated six times. The nutrient addition level was based on the rate of addition of P to achieve sufficient P for the cultivar of barley used in this experiment on this soil and was derived from previous studies (George et al., 2011). Specifically, the nutrient treatments were designed to be equivalent to the input of 20 t (fresh weight) green manure per hectare of soil to 25 cm depth (3.0 x 10^6 kg soil) and were approximately equivalent to the addition of 50 kg P ha^{-1} and 250 kg N ha^{-1} when applied as a mixed green manure at the 100% dose rate. This provides a level of P adequate for plant growth, but due to the natural P:N ratio of the material provides a high rate of N fertilisation. All chopped biomass, ground fertiliser granules (NPK 22-4-14) and chemicals (NH_4NO_3 and KH_2PO_4)), were mixed thoroughly with soil in a cement mixer for 15 minutes (75 rpm). Control soils were given the same mixing treatment without any soil amendments. Filter papers (90 mm diameter - Whatman International Ltd, Maidstone, UK), were placed in the bottom of 1 L (13 cm diameter, 11 cm depth) plastic pots to prevent soil loss and the pots were filled with 1 kg of the variously prepared soils. The pots were watered to 80% field capacity (equivalent to 40-50 kPa water potential) as determined by gravimetric water content and maintained at this level by daily watering to weight with deionised water. Pots were incubated in the glasshouse for 28 days prior to planting.

Plant growth

Seeds from a commonly grown local barley variety (*Hordeum vulgare* cv. Optic) were germinated in 90 mm diameter Petri dishes containing 0.5% distilled water agar [1 g of BDH Agar (VWR, UK) per 200 ml distilled water]. At four days post germination, seedlings were planted (one per pot) in the pots

containing the incubated soil treatments. Pots were placed on the bench in the glasshouse in a randomised design and were rotated regularly to reduce the effects of environmental gradients. The glasshouse temperature was maintained between 18 °C during the day and 14 °C at night with a 16 h day length and a minimum light intensity of 200 W m^{-2} was ensured by supplementary lighting. Daily watering to weight with deionised water was performed in order to maintain field capacity at 80%. Leaf samples of newest fully expanded leaves were taken when plants reached flag leaf stage (GS49 – flag leaf unfurled and first awns visible – (Tottman, 1987)) and stored at -20°C for shoot P, N, and C analyses, described below. Watering ceased when the peduncles of more than half the plants had turned yellow and plants were allowed to dry out to maturity. Mature barley plants were harvested and above ground biomass and grain weight were measured.

Plant tissue analysis

Number of tillers, above-ground biomass (biomass), number of heads, head dry weight, and grain weight (yield) were recorded. The number of tillers for each replicate was counted before the harvested plant material was oven-dried at 70 °C for 4 days after which plant dry weight and head dry weight were recorded. Seeds were detached from the heads, cleaned with the aid of an Agriculex CB-1: Column Blower with an acrylic trash catcher (Agriculex, Guelph, Canada) and weighed.

The frozen flag leaf samples were freeze dried for 48 hours using a Sciquip CHRIST Alpha 1-2 freeze dryer (SciQuip, Shropshire, UK) and then ground using a QIAGEN TissueLyser II (QIAGEN, Hilden, Germany). Nitrogen and C concentrations in plant tissues were determined by the combustion (plant flour of 2-5 mg) in pure oxygen under static conditions using a CE440 Elemental Analyser (Leeman Labs, NH, USA). Tissue P concentrations were determined on the powdered leaf material (50 mg) which was digested in 15.8 M nitric acid HNO3 (Aristar grade, VWR International, Poole, UK) for 20 min at 180ºC and thereafter oxidised in 1 ml of 30% hydrogen peroxide (H2O2) in closed vessels using a MARSXpress microwave oven (CEM, Buckingham, UK). After digestion, the solution was made up to a final volume of 50 mL with dH2O. Phosphate was determined using Malachite Green reagent (Irving and Mclaughlin, 1990) using a Multiskan GO UV-Vis spectrophotometer (Thermo Scientific, MA, USA) at 620 nm. Nutrient accumulation in above ground tissue of barley plants and individual bufferstrip species was calculated by multiplying the shoot biomass with the nutrient

concentration in the sample. This was then used to assess the relative effect of soil treatment on barley nutrition or species on the ability to uptake and accumulate nutrients.

Screening riparian buffer strip species to establish potential for use as green manure (Experiment 2)

A selection of 33 species of annual and perennial plants which can be found present in Scottish buffer strips, with growth habits that were considered useful for green manure production and were available to purchase as seed from merchants, were grown in pots containing a sand/compost mix under high P and N input conditions. Seeds of the individual species were pre-germinated in petri dishes containing 0.5% distilled water agar [1 g of BDH Agar (VWR, UK) per 200 ml distilled water] then transferred to 1 kg pots (one plant per pot) containing 50:50 sand, compost mix. Five replicates of each species were planted. Pots were watered daily with 30 ml of a solution containing 32 mg P L^{-1} and 320 mg NO_3 L^{-1}, a dose considered to be equivalent to the influx of nutrients to a buffer strip under nutrient excess conditions (Stutter, personal communication). All other nutrients were supplied weekly at the following concentrations 2 mM KNO_3, 1 mM $MgSO_4$, 80 µM FeEDTA, and micronutrients [30 nM H_3BO_3, 6 µM $CuSO_4$, 6 µM $MnSO_4$, 0.6 µM $ZnSO_4$, 42 nM NH_4Mo_7, 12 µM $Co_4(NO_3)_2$] at a rate of 25 mL per pot per week. The newest fully expanded leaf was sampled just prior to harvest, which occurred at the onset of flowering, and stored at -20ºC for shoot P analysis. Tissue P concentrations were determined as described in Experiment 1. Plants were then harvested at flowering stage by cutting at the pot surface and the above ground plant material was oven dried at 70ºC for 4 days. Dried plants were then weighed and the above ground biomass recorded. Phosphorus accumulation (mg P $plant^{-1}$) was calculated as the product of biomass and P concentration in the shoot tissue.

Effectiveness of green manure from specific riparian plant species (Experiment 3)

In a follow on study to investigate the potential of individual species in their effectiveness as green manures, seven species were selected from the screen of riparian buffer strip species (Experiment 2). The selection included four monocot species (*Festuca rubra, Lolium perenne, Agrostis canina, Cynosurus cristatus*) and two dicot species (*Myosotis arvensis, Trifolium repens*), which provided a range in capabilities to accumulate nutrients under excess input conditions. In addition, one crop plant (*Hordeum vulgare*) was included. These green manure species were grown from seed in standard compost in 10 L (27.5 cm diameter, 22 cm depth) plastic pots, in a glasshouse for 12 weeks under the

same controlled glasshouse conditions as described in Experiment 1. Harvested plant material from either field or glasshouse was chopped into 1-10 mm pieces using scissors prior to use as green manure. Soil preparation, plant growth and plant tissue analysis were all as described in Experiment 1. The dose rates of green manure and integrated nutrient additions can be seen in supplemental Table S2.

Nutrient release to soil upon decomposition of riparian buffer strip species (Experiment 4)

A subset of the species screened in Experiment 2, which had various nutrient accumulation capabilities, were selected to assess nutrient release to soil. The dicotyledonous species, *Myosotis arvensis* and *Trifolium repens*, and the monocotyledonous species *Festuca rubra*, and *Lolium perenne* were grown for 12 weeks from seed in standard compost in 10 L (27.5 cm diameter, 22 cm depth) plastic pots, under the same growth conditions described in Experiment 1.

The mature green manure plant species were harvested and the fresh material was chopped to 1-10 mm size pieces and this homogenized tissue analysed for C, N and P content as described for Experiments 1. Soil, categorised in Experiment 1, was mixed to form treatments detailed in Supplemental Table S2 as follows. Specifically one kg of topsoil was amended as follows: 1) non-fertilised (Control), 2) amended with 20 t ha^{-1} equivalent green manure derived from the four individual species, 3) amended with 20 t ha^{-1} equivalent green manure, derived from an even mix of all four species. 4) amended with NPK (22-4-14) fertiliser (delivering equivalent N and P as the green manure treatment – 50 kg P ha^{-1} and ~~250~~ between 100 and 500 kg N ha^{-1}, dependent on the N:P ratio of the specific source of green manure). Each treatment was replicated five times. Soils were incubated in pots in a greenhouse, under the conditions described above for plant growth (Experiment 1), and were placed on the bench at random in order to avoid any potential environmental gradients. Soils were watered daily to weight with deionised water to maintain ~~equivalent of 40-50 kPa water potential~~80% field capacity. Soil treatments were left to incubate for 10 weeks prior to sampling. Soil core samples were taken from the pots at the end of the incubation period. Samples were transferred into 50 mL tubes and stored at 4 ºC until analysis.

Soil P was determined using the sodium bicarbonate ($NaHCO_3$) extraction method (Olsen et al., 1954). In 50 mL centrifuge tubes, 0.5 g of soil and 5 mL of 0.5M $NaHCO_3$ were shaken for 60 minutes. The tubes were then centrifuged for 5 minutes at 5100 rpm. After centrifugation the supernatant was

stored at 4°C until analysis. Phosphate was determined using Malachite Green reagent, described in Experiment 1. Ammonia-N, NO_3-N, and total organic carbon (TOC) were measured using the potassium chloride (KCl) extraction method. To prepare the extract, 5 g of soil and 25 ml of 1 M KCl were placed in 50 mL centrifuge tubes and tubes were shaken (45 rpm) for 45 minutes and then centrifuged for 5 minutes at 5100 rpm. The supernatant was stored at 4 °C until analysis. Ammonia-N and NO_3-N were determined spectrophotometrically using a Konelab Aqua 20, Discrete Analyser, (Thermo Electron Corporation, Finland). Total organic carbon was determined using an OI Analytical 1010 analyser (OI Analytical, TX, USA).

Statistical analysis

Genstat v12.1 (VSN International, 2009) was used for all statistical analyses. For all experiments, design was completely randomised. For data that were skewed, a logarithmic transformation of the variable was used. After performing analysis of variance (ANOVA), graphs illustrating residuals versus fitted values were also plotted for every variable before and after log transformation in order to check that the transformed variables were normally distributed. All data are presented as the mean of either five or six replicates with standard error of the mean presented as error bars on graphs. Where differences within a treatment main effects and interactions are statistically significant (p<0.05), a LSD is also presented and used to compare individual treatments where appropriate.

Results

Comparison of the effectiveness of green manure from bufferstrips (Experiment 1)

Soil amendments had a significant (P<0.001) effect on both biomass (Figure 1a) and yield (Figure 1b) compared to the control. For biomass (Figure 1a), dry weights ranged from 0.58 g for soil amended only with N fertiliser, to 6.62 g for soil amended with standard NPK fertiliser. Fertiliser produced significantly (P<0.001) greater biomass than all other treatments. No significant effects were found for green manure or INM treatments compared to the control, with the exception of the green manure +NPK treatment, which produced significantly (P<0.001) greater biomass when the green manure was from Balruddery. For grain yield (Figure 1b), the P fertiliser treatment was significantly (P<0.001) greater than all other treatments, while the green manure +NPK integrated treatment yielded equivalent to the standard NPK fertiliser for Balruddery green manure at 1.95 and 1.88 g grain plant^{-1},

respectively. Grain yield was significantly (P<0.001) less for green manure +N for Balruddery green manure, while plants grown in soil amended with N fertiliser alone failed to produce any grain yield.

Other growth parameters (Table 1) also demonstrated a similar pattern with nutrient source, with soil amendments having a significant (P<0.001) effect on number of tillers and heads, and head dry weight. Fertiliser treatments where P and NPK were added as an inorganic fertiliser, or where green manure from Balruddery was added with inorganic NPK, had significant (P<0.001) effects. None of the other fertiliser treatments were significantly different from the control.

Phosphorus accumulation in the plants was significantly (P<0.001) greater than the unfertilised control only where inorganic P fertiliser was added, and was significantly (P<0.001) less when inorganic N fertiliser was added. In contrast, N accumulation in plants was significantly (P<0.001) greater than the unfertilised control where inorganic NPK fertiliser was added and with both green manure sources when they were integrated with an addition of inorganic N, in the form of NPK for Balruddery or N alone for Tarland green manure. Again the organic amendments alone did not show any significant difference from the control.

Screen of riparian buffer strip species (Experiment 2)

The selection of 33 typical riparian buffer strip species demonstrated a large significant (P<0.001) variation in biomass response (90-fold) and P accumulation (38-fold) when treated with excess inputs of N and P (Figure 2). Biomass (above ground dry weight) ranged from 0.09 g for *Juncus effusus* to 41.58 g for *Holcus mollis.* P accumulation ranged from 1.38 mg P plant^{-1} for *Cardamine pratenses* to 52.73 mg P plant^{-1} for *Agrostis canina.* The species order for P accumulation was not correlated (N.S.) with that for biomass.

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Effectiveness of green manure from specific riparian plant species (Experiment 3)

There were no significant differences between the yield (dry grain weight) of barley plants grown in soil amended with green manures derived from the individual buffer strip species (Figure 3) nor for plants growing in INM treatments, when compared to a no fertiliser control. No significant differences were found between the green manure only and INM treatments.

Nutrient release to soil upon decomposition of riparian buffer strip species Experiment 4

The nutrient characteristics of the specific riparian buffer strip species assessed are contained in Table 2. The greatest concentrations of N were found in *Trifolium repens* (56.7 mg g^{-1}) whilst the greatest concentrations of P were found in *Myosotis arvensis* (11.7 mg g^{-1}). *Festuca rubra* exhibited both the greatest C:N and C:P (24 and 94 respectively) while *Trifolium repens* had the smallest C:N (8) and *Myosotis arvensis* the smallest C:P (31).

Nutrient concentrations of the soils following the addition and incubation of single species green manures show some significant (P<0.001) differences for NH_4-N, NO_3-N and TOC (Table 3). Ammonium was greater in soils amended with green manure from *Festuca rubra*, *Trifolium repens,* *Lolium perenne* and the 4 species mixed compared to the control, the NPK fertiliser and *Myosotis arvensis* treatments. For NO_3-N, the NPK and *Trifolium repens* treatments were significantly (P<0.001) greater than all other treatments at 33.39 mg kg^{-1} and 31.435 mg kg^{-1}, respectively. Total Organic Carbon was significantly (P<0.001) greater for the *Lolium perenne* and 4 species mix than all the other treatments. P concentrations however, were not significantly affected by the various nutrient sources.

Discussion

Taken together our data on plant growth, yield and nutrition suggest there is no benefit to crop plants in nutrition or yield when grown with green manures taken from buffer strip systems relative to unfertilised soils and that inorganic fertilisers are a ~~far~~ superior source of nutrients in these soils under the artificial conditions of this pot experiment. Our results were similar to those of Baggs et al., (2000), where cover crop incorporation as a green manure in field soils from NE Scotland had no impact on yield of oats and are also consistent with a range of other studies in temperate regions with other crop species (Jackson et al., 1993; Knott, 1996; Elfstrand et al., 2007). This is, however, in contrast to a number of studies in the tropics in low input systems, which see benefits in crop growth and yield to the addition of green manure (Gachengo et al., 1996; Fischler et al., 1999; Jama et al., 2000; Cobo et al., 2002a) and in studies on sandy soils in temperate regions (Olesen et al., 2007; 2009). The general lack of response in yield to the addition of green manure in temperate systems may not be as simple as temperature affecting decomposition and release of nutrients, as clover green manures have been shown to decompose when incorporated even in the low temperatures (Breland, 1994), but is likely to do with the general high fertility of these soils. It could also be due to inherent

differences in species used in green manure systems in the tropics compared to temperate regions, with a greater propensity of legume species used in the former leading to greatly improved N nutrition in tropical green manure systems.

For both biomass production and yield of barley plants grown with a range of inorganic fertiliser, green manure amendments and integrated nutrient management additions, were only significantly greater from the unfertilised control when NPK or P was added as an inorganic fertiliser alone or when the green manure from Balruddery was integrated with NPK (Figure 1). The data again suggest that the plants only achieve their greatest growth when P deficiency is relieved. In contrast, the biomass of barley plants was reduced when inorganic N was added alone and the yield of plants was reduced when N was added as an integrated addition with both green manure sources (Figure 1). The biomass and yield reductions with N addition could be explained by a strong immobilisation by microbes of P in the these conditions leading to even greater P deficiency, which is partially backed-up by the significant decline in P accumulation seen in the N fertiliser treatment. It is, however, apparent that green manures can partially replace some of the fertiliser inputs when they are added in an integrated manner with NPK (Figure 1), but it is important to note that the yield of the NPK treatment is not achieved and that positive responses were only observed with the green manure from Balruddery, which is likely to have different quality characteristics compared to the Tarland green manure (Table S1). Balruddery green manure was harvested in winter in comparison to summer for the Tarland green manure, which would likely be detrimental to the quality of the material as it is likely to senesce and remobilise nutrients in winter (Baggs et al., 2000). It is also important to note that the thorough mixing of nutrient resources in this controlled environment pot experiment is very different to the agronomy of adding fertilisers in the field where nutrients will tend to accumulate in the surface and potentially become more easily available to plants and microbes, so the full potential of the green manure may not be realised in this experiment.

When a range of plant species common to riparian buffer strips in Scotland were grown in conditions of excess N and P supply, a large range of biomass and P accumulation responses were seen with greater than 90-fold difference in biomass production and 38-fold difference in P accumulation observed (Figure 2). This is consistent with previous studies that have demonstrated large differences in nutrient accumulation abilities between different plant species (Broadley et al., 2004). We suggest

that species with large biomass and nutrient accumulation (e.g. *Argrostis canina*) would be optimal for green manure systems, compared to those with small biomass and nutrient accumulation (e.g. *Myosotis arvensis*). Use of these selected species would go some way to mitigating the impact of nutrient accumulation in buffer strips and reduce the potential of these to act as a source of polluting nutrients (Borin et al. 2005). Based on observations made in this study, we are able to ~~calculate~~ estimate that the species accumulating the largest amounts of P would be able to sequester around 10% of all the P accumulating in a typical buffer strip of eastern Scotland in a given year, while the poorest accumulator would sequester less the 1%.

Unsurprisingly, the legume *Trifolium repens* had the smallest C:N, while the grasses *Festuca rubra* and *Lolium perenne* had the largest C:N. The grasses also had the largest C:P, while the *Myosotis arvensis* had a much smaller C:P than the other species. Our data for C:N are consistent with Baggs et al. (2000), who also showed smallest ratios for legumes, followed by dicots and then grasses. Our data for C:N were also similar to those found for tropical green manure species (Cobo et al., 2002a), but the C:P ratios here were an order of magnitude less than the tropical species. These data would suggest *Trifolium repens* should be the best source of N to soil upon decomposition, while *Myosotis arvensis* should best supply P. *Festuca rubra* had the largest C:N and C:P (Table 2) and would therefore suggest that the addition of this as a green manure source would be the most likely to lead to immobilisation of the nutrients in the soil and reduced plant availability.

The addition of green manure amendments from the specific species had no impact on the P availability in the soil, but neither did the addition of NPK (Table 3). In contrast, the soil NH_4 levels were increased by all species specific organic amendments except the *Myosotis arvensis* and when all species were added together (Table 3), while, NO_3 was only increased in the soil with the addition of NPK and the *Trifolium repens* green manure. In the case of P, none of the amendments had the predicted effect on the availability of P in the soil expected due to their C:P ratio. It was clear that the addition of the leguminous green manure had a large impact on the availability of N increasing both NH_4 and NO_3. This result was also seen by a number of other authors (Diekman et al., 1993; Cobo et al., 2002a) and has been attributed to enhanced microbial activity in soils amended with legume biomass (Elfstrand et al., 2007). The larger C:N of the other green manure amendments may explain their limited impact on NO_3 availability (Cobo et al., 2002b) and all but *Myosotis arvensis* enhanced

399 NH_4 concentration (Table 3). The lack of impact of *Myosotis arvensis* on the availability of nitrogen
400 may be explained by its exceptionally small C:P, which may force any liberated N to be rapidly
401 immobilised to satiate the microbes need for N to catalyse the decomposition of C and mineralisation
402 of P (Lovell and Hatch., 1998; Cobo et al., 2002b). Likewise, *Myosotis arvensis* was the only green
403 manure amendment which did not increase TOC, suggesting the large P content of this was driving C
404 immobilisation by soil microorganisms.

405 The impact of green manure quality (e.g. soluble C, lignin and polyphenol contents) can have a large
406 effect on the balance between mineralisation or immobilisation of nutrients when added to soil and
407 there are also many interacting factors such as soil characteristics, temperature and method and
408 timing of incorporation (Oglesby et al., 1992; Palm et al., 1988; Baggs et al., 2000; Jama et al., 2000,
409 Cobo et al., 2002a). The interaction between quality and timing is critical. Previous research has
410 shown that the greatest benefits of a green manure with a large C:N ratio occur if the crop is sown
411 with a delay, while green manures with small ratios are best followed immediately by the crop (Rayns
412 and Lennartson, 1995). If this were the case here, grass species green manure should have been
413 more effective at promoting crop growth than the other green manure due to their large C:N ratio and
414 the delay between planting and incorporation, but there were no differences in yield based on green
415 manure type (Figure 3).

416 Despite the selected species having greatly contrasting abilities to accumulate nutrients and observed
417 differences in tissue quality and the amount of nutrient they release to the soil upon decomposition
418 (Tables 3 and 4), there was no impact of using them as green manures on yield (Figure 3). This again
419 suggests that green manure in general is not a useful source of nutrients for agricultural crops in this
420 soil, under these artificial controlled conditions. In fact, it appears that green manures have
421 complicated effects on nutrient availability that could be a consequence of their impact on the balance
422 between nutrient immobilisation and mineralistion associated with their tissue quality. Similar
423 conclusions have also been reached for high intensity tropical systems (Becker et al., 1995), where it
424 was suggested that such green manure systems were better suited to marginal soils with poor
425 nutrient availability.

426 The main conclusion of this study is that despite selecting specific species of plants with an ability to
427 accumulate large amounts of nutrients in their tissue, their use as green manures was not effective.

This further suggests that the potential additional benefit of promoting these species in buffer strip systems with a view to using this biomass as a source of nutrients is not supported by this research. There are, however, a range of important variables that should be tested before this technology is dismissed ~~totally~~ for intensively managed conventional temperate systems, with already nutrient replete soils. Variation in plant tissue quality should be considered, as should timing of cutting of the material and management of application. It is also critical to test whether the producing area of green manure in the landscape has the capacity to provide enough biomass to make a significant impact on the nutrient requirement of the receiving area, results here suggest that this would be unlikely. The potential of the system should be monitored over a number of seasons and over a number of years of agronomically appropriate application, as it has been seen that N uptake in wheat crops was only seen in subsequent years in Canadian systems (Janzen et al., 1990). It is also important to remember that there are a number of other benefits to increasing the organic matter content of soils by adding green manure, such as improving soil physical properties and carbon contents. These additional benefits and the general theory of using buffer strip material as green manure should be tested under field conditions to evaluate this technology effectively.

Acknowledgements

The authors would like to thank Euan Caldwell and the estates staff at the James Hutton Institute for provision of soil and maintenance of riparian buffer strips at the institute's farm. Richard Gwatkin assisted with fieldwork in the Tarland catchment and we thank the MacRobert Trust Estate for access to sites around Tarland. The Balruddery buffers are based at the Centre for Sustainable Cropping long-term platform, funded through Scottish Government Underpinning Capacity, while the experimental work was performed with the financial support of the Rural & Environment Science & Analytical Services Division of the Scottish Government and through a scholarship held by CK from the Scottish Funding Council and the University of Edinburgh.

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566

567 **Tables**

568 Table 1: Growth, development [No. tillers, No. heads and Head dry weight (DW)] and nutrient
569 concentration (con) and accumulation (acc) parameters for C, N and P in tissue of barley grown in soil
570 amended with a range of green manures (GM), integrated nutrient management (INM), fertiliser and
571 control treatments (Experiment 1). Nutrient accumulation is the product of the concentration of the
572 nutrient in the tissue and the tissue biomass. Data are the means of six replicates and level of
573 significance (***, $p<0.001$) and least significant differences (LSD) were generated using ANOVA.

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	Tillers (n)	Heads (n)	Head DW g pot^{-1}	P con mg g^{-1}	P acc mg plant^{-1}	C con mg g^{-1}	C acc mg plant^{-1}	N con mg g^{-1}	N acc mg plant^{-1}
Zero Control	3.2	1.5	1.3	2.8	8.5	411.7	1279.0	18.3	56.6
Balruddery GM	2.0	1.5	1.4	2.9	8.1	407.0	1100.0	18.6	50.8
Balruddery GM +N	3.0	1.0	0.4	0.7	2.2	412.6	432.0	24.8	77.3
Balruddery GM +P	2.2	1.8	1.5	4.0	10.8	407.1	1130.0	17.8	49.7
Balruddery GM +NPK	5.2	3.0	2.5	2.5	13.7	416.7	2213.0	20.2	116.9
Tarland GM	2.7	2.2	1.7	2.6	8.4	409.7	1530.0	16.5	61.4
Tarland GM +N	4.2	1.3	0.8	3.1	13.8	412.6	1441.0	53.5	174.5
Tarland GM +P	1.8	1.2	1.2	4.4	9.0	407.4	843.0	17.4	35.2
Tarland GM +NPK	2.5	1.5	1.5	2.6	7.9	415.1	1273.0	17.1	52.5
Fertiliser +N	1.0	-	-	3.2	1.8	418.6	246.0	54.4	32.8
Fertiliser +P	4.2	2.7	2.4	3.8	16.9	412.8	1908.0	19.9	91.0
Fertiliser +NPK	5.2	3.5	3.4	2.1	13.6	419.6	2787.0	18.7	128.8
Level of significance	***	***	***	***	***	***	***	***	***
LSD	2.9	1.1	1.0	1.0	6.5	5.0	897.0	3.8	55.9

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578 Table 2: Nutrient concentration and stoichiometric mass ratio of selected buffer strip species grown
579 for 12 weeks in compost with excess nutrient addition (Experiment 4). Data represent a single point
580 analysis of a bulked sample of material used in subsequent studies.

	C (mg g^{-1})	N (mg g^{-1})	P (mg g^{-1})	C:N	C:P
Myosotis arvensis	369	20.3	11.7	18	31
Festuca rubra	413	17.3	4.4	24	94
Trifolium repens	432	56.7	5.8	8	75
Lolium perenne	402	19.8	4.9	20	82

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583 Table 3: Available P, NH_4 and NO_3 and Total organic carbon (TOC) concentrations of soils following
584 addition and 70 days decomposition of green manures made up of specific species or an even mix of
585 the four species (Experiment 4). These were compared to a no fertiliser and a NPK control. Data are
586 the mean of 5 replicates and level of significance (- = not significant; *** = p<0.001) and least
587 significant differences (LSD) were determined using ANOVA.

588

Nutrient source	**P (mg kg^{-1})**	**NH_4-N (mg kg^{-1})**	**NO_3-N (mg kg^{-1})**	**TOC (mg kg^{-1})**
Control	28.4	0.4	18.1	20.7
NPK	28.4	0.4	33.3	20.9
Myosotis arvensis	28.7	0.4	14.1	23.1
Festuca rubra	27.9	0.7	14.2	24.5
Trifolium repens	29.4	0.8	31.4	26.5
Lolium perenne	27.4	0.9	14.1	28.0
4 Species mix	27.0	0.8	18.7	28.9
Level of significance	-	***	***	***
LSD	2.5	0.2	7.9	6.5

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Figure Legends

Figure 1: a) Biomass production and b) yield of barley grown in soils amended with green manure and green manure integrated with nitrogen (+N), phosphorus (+P) and fertiliser (+NPK) compared to a NPK (fertiliser) and no addition control (Experiment 1). All soils, except the no addition control, received the equivalent of 50 kg P ha^{-1} and 250 kg N ha^{-1} (See Table S2). Green manures were sourced from existing conventionally managed buffer strips at Balruddery and Tarland in the NE of Scotland. The data are the mean of six replicates and the error bars represent +/- one standard error of the mean. A LSD for the interaction is presented on the graph to allow comparison between treatments.

Figure 2: a) Biomass production and b) P accumulation of a range of common buffer strip species grown for 12 weeks in a sand compost mix amended with N and P to levels typical of a riparian buffer strip, such that these nutrients are in excess of requirement (Experiment 2). Red bars represent the species which were selected for further study on impact on plant growth and supply of nutrients to soil. The data are the mean of six replicates and the error bars represent +/- one standard error of the mean. A LSD for the main effect of species is presented on the graph to allow comparison between species.

Figure 3: Yield of barley after growth in soil amended with green manure from specific individual species typical of riparian buffer strips in Scotland and the same sources of green manure when applied in an integrated nutrient management (INM) system where half of the required nutrient is provided as inorganic sources (Experiment 3). All soils received the equivalent of 50 kg P ha^{-1} and ~~250~~ between 100 and 500 kg N ha^{-1} (See Table S2), dependent on the N:P ratio of the material added. Plants were grown for 14 weeks in controlled conditions and compared to an unfertilised control. The data are the mean of six replicates and the error bars represent +/- one standard error of the mean. No LSD is presented as there were no significant differences observed.

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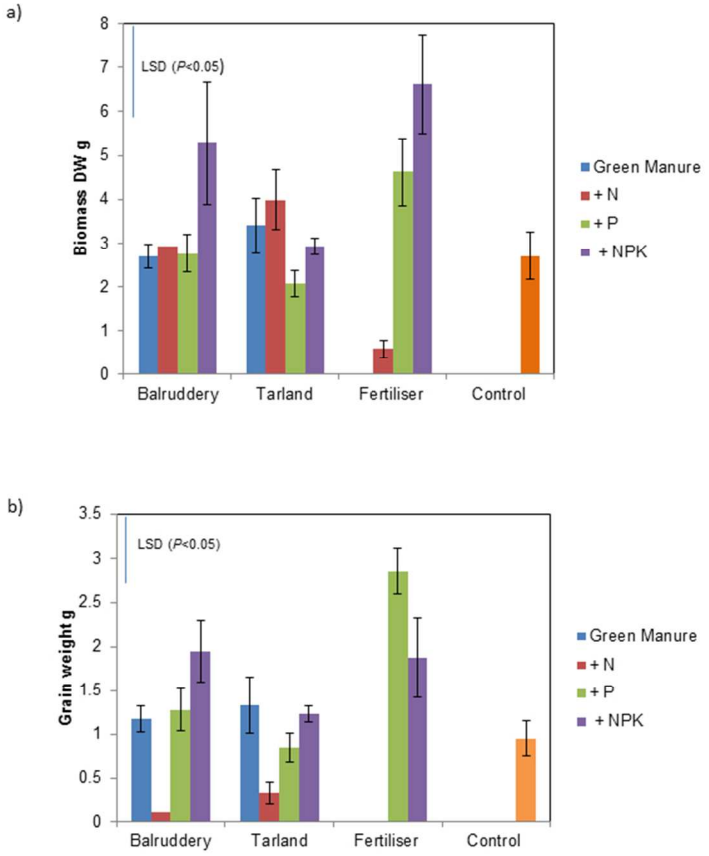

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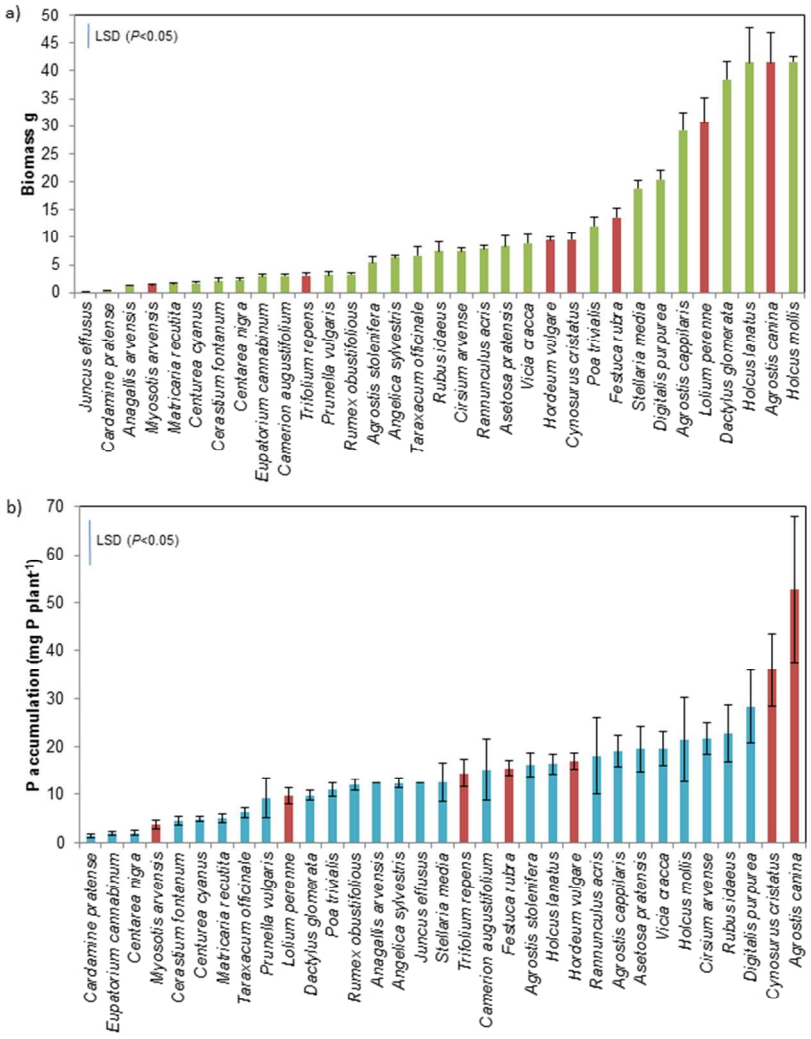

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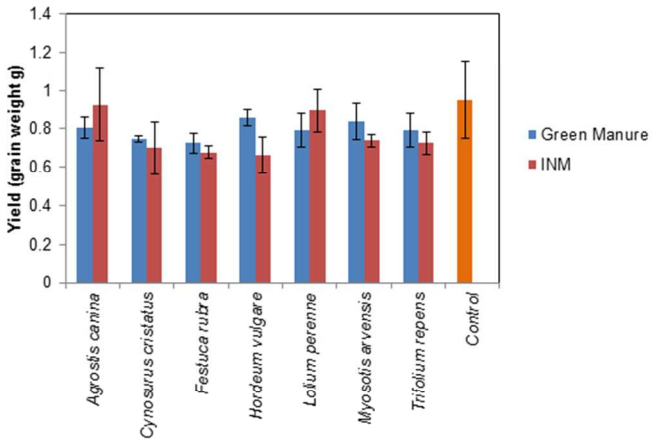

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Supplemental Tables

Table S1: List of dominant species and orders of species found in riparian buffer strips from Tarland and Balruddery in NE Scotland. The total number of species and the total number of species in each order (in parentheses) are presented. The list of 33 species selected to represent typical riparian buffer strips is also presented in the last column.

Buffer strip source	Balruddery	Tarland	Species screened
No. species	78	74	Experiment 2
Dominant species	*Aegopodium podagraria* *Anthriscus sylvestris* *Arrenatherum elatius* *Chamaenerion angustifolium* *Cirsium arvense* *Crataegus monogyna* *Dactylis glomerata* *Elytrigia repens* *Epilobium angustifolium* *Equisetum sp* *Filipendula ulmaria* *Fumaria sp* *Galium aparine* *Heracleum sphondylium* *Lolium perenne* *Matricaria discoidea* *Matricaria recutita* *Myosotis arvensis* *Plantago major* *Poa annua* *Poa trivialis* *Polygonum aviculare* *Rubus fruticosus* *Rubus idaeus* *Rumex sp* *Senecio vulgaris* *Taraxacum officinalis* *Trifolium repens* *Tripleurospermum inodorum* *Urtica dioica*	*Agrostis stolonifera* *Dactylis glomerata* *Festuca rubra* *Holcus lanatus* *Holcus mollis* *Juncus effusus* *Phragmites australis* *Poa trivialis* *Anthriscus sylvestris* *Cirsium arvense* *Filipendula ulmaria* *Galium aparine* *Ranunculus repens* *Rumex obtusifolios* *Urtica dioca*	*Agrostis canina* *Agrostis cappilaris* *Agrostis stolenifera* *Anagallis arvensis* *Angelica sylvestris* *Asetosa pratensis* *Camerion augustifolium* *Cardamine pratense* *Centarea nigra* *Centurea cyanus* *Cerastium fontanum* *Cirsium arvense* *Cynosurus cristatus* *Dactylus glomerata* *Digitalis purpurea* *Eupatorium cannabinum* *Festuca rubra* *Holcus lanatus* *Holcus mollis* *Hordeum vulgare* *Juncus effusus* *Lolium perenne* *Matricaria recutita* *Myosotis arvensis* *Poa trivialis* *Prunella vulgaris* *Rannunculus acris* *Rubus idaeus* *Rumex obustifolious* *Stellaria media* *Taraxacum officinale* *Trifolium repens* *Vicia cracca*
Orders	Apiales (2) Aquifoliales (1) Asparagales (1) Asterales (14) Boraginales (3) Brassicales (1) Caryophyllales (6) Dennstaeditales (1) Dipsacales (1) Ericales (1) Fabales (3) Fagales (3) Gentianales (1) Lamiales (6) Malphighiales (2) Malvales (1) Myrtales (1) Pinales (1) Poales (16) Ranunculales (1) Rosales (11) Spinales (1)	Apiales (4) Asparagales (1) Asterales (9) Boraginales (2) Brassicales (1) Caryophyllales (5) Dennstaedtiales (1) Dipsiacales (1) Ericales (1) Fabales (5) Gentianales (2) Lamiales (11) Maplighiales (2) Myrtales (2) Poales (17) Ranunculales (3) Rosales (7)	

Table S2: Combination of treatments used to demonstrate the effect of green manures of different sources when supplying nutrients alone or in combination with inorganic fertilisers in integrated nutrient management systems when compared to unfertilised controls and fertiliser treatments including N and P alone and NPK.

	Nutrient Addition (% dose)			
Soil Treatment	**N**	**P**	**NPK**	**Green Manure (20t ha^{-1})**
(100 % dose rate)	(250 kg N ha^{-1})	(50 kg ha^{-1})	(250 kg N ha^{-1}; 50 kg P ha^{-1})	(250 kg N ha^{-1}; 50 kg P ha^{-1})
Control	-	-	-	-
Balruderry Green Manure	-	-	-	100
Tarland Green Manure	-	-	-	100
Balruddery Green Manure +N	50	-	-	50
Tarland Green Manure +N	50	-	-	50
Balruddery Green Manure + P	-	50	-	50
Tarland Green Manure +P	-	50	-	50
Balruddery Green Manure +NPK	-	-	50	50
Tarland Green Manure +NPK	-	-	50	50
A. canina Green Manure	-	-	-	100
C. cristatus Green Manure	-	-	-	100
F. rubra Green Manure	-	-	-	100
H. vulgare Green Manure	-	-	-	100
L. perenne Green Manure	-	-	-	100
M. arvensis Green Manure	-	-	-	100
T. repens Green Manure	-	-	-	100
A. canina Green Manure +NPK	-	-	50	50
C. cristatus Green Manure +NPK	-	-	50	50
F. rubra Green Manure +NPK	-	-	50	50
H. vulgare Green Manure +NPK	-	-	50	50
L. perenne Green Manure +NPK	-	-	50	50
M. arvensis Green Manure +NPK	-	-	50	50
T. repens Green Manure +NPK	-	-	50	50
Fertiliser N	100	-	-	-
Fertiliser P	-	100	-	-
Fertiliser NPK	-	-	100	-

The nutrient treatments were designed to be equivalent to the input of 20 t green manure ha^{-1} of soil to 25cm depth and were on average the equivalent of the addition of 50kg P ha^{-1} and 250kg N ha^{-1} when applied at the 100% dose rate.