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

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**Is green manure from riparian buffer strip species an effective nutrient source for crops?**

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

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10

11 **Abbreviations**

12 acc, accumulation

13 ANOVA, analysis of variance

14 C, carbon

15 con, concentration

16 DW, dry weight

17 ghg, greenhouse gas emissions

18 GM, green manure

19 INM, integrated nutrient management

20 K, potassium

21 LSD, least significant difference

22 N, nitrogen

23 NH<sub>4</sub>, ammonium

24 NO<sub>3</sub>, nitrate

25 P, phosphorus

26 PO<sub>4</sub>, phosphate

27 TOC, total organic carbon

## 28 Core Ideas

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

## 35 Abstract

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

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## 56 Introduction

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

62 The accumulation and subsequent mobilisation of large concentrations of nitrogen (N) and phosphate  
63 ( $\text{PO}_4$ ) in agricultural soils is a major contributor to surface water pollution and an indicator of  
64 imbalance in agricultural systems world-wide (Condrón et al., 2013). This is particularly true in  
65 Europe, the United States, and Asia where agricultural intensification and profligate use of manures  
66 and chemical fertilisers has increased soil nutrient stocks beyond crop requirement (Rosemarin and  
67 Ekane, 2016). As the mismanagement of soil continues to push the global boundaries of nutrient  
68 sustainability (Steffen et al., 2015), new approaches are needed to more effectively manage crop  
69 nutrition.

70 Nutrient utilisation in agriculture could be improved by recycling nutrients within the agricultural  
71 landscape. The transfer of nutrient rich organic materials from zones where nutrients accumulate to  
72 zones where nutrients are required for production could contribute to such a solution (Jama et al.,  
73 2000; [Mankin et al. 2007](#)). This represents a process of 'closing the loop' of nutrient use. The use of  
74 green manure, in which plant biomass is produced specifically to provide an input of nutrients to  
75 crops, could offset some of the required fertiliser input. This is a relatively common practice in low-  
76 input systems in the tropics (Sharma and Mitra, 1988; Becker et al., 1995; Dreschel et al., 1996;  
77 Fischler et al., 1999; Gachengo et al., 1999; Jama et al., 2000; Cobo et al., 2002a) and in organic  
78 systems in temperate regions (Baggs et al., 2000, Olesen et al., 2007, 2009), but is rare in temperate  
79 intensive agricultural systems.

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

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

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

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

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

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

## 129 **Materials and Methods**

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

#### 131 Green manure preparation

132 In an initial experiment, plant material used as green manure, was collected from two different riparian  
133 buffer strip sites in Scotland. The first was Balruddery farm (56°29'10", 3°8'8", elevation 70-124 m), a  
134 170 ha arable farm near the James Hutton Institute, 10 km west of Dundee, Scotland. The second  
135 was from the Tarland catchment in Aberdeenshire in northeast Scotland (centred on 57°53' N, 2°00'  
136 W, elevation range 145-298 m). Plants from Tarland were harvested during the second week of July  
137 2013 from three sites and stored at -20 °C until the experiment was set up in December 2013. Plants  
138 from Balruddery were harvested in December 2013. ~~The average nutrient concentrations in buffer strip~~  
139 ~~green manure material were 2.6, 12.7 and 183.3 mg kg<sup>-1</sup> dry weight for P, N and C, respectively, and~~  
140 ~~these were used to calculate biomass addition rates later.~~ The species composition of the different  
141 buffer strips can be seen in supplemental Table S1. There were >70 species present at both sites,

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

#### 145 Soil Preparation

146 Topsoil (0-10 cm depth) of a typical arable soil of the region, characterised as sandy silt loam,  
147 Balrownie series, Cambisol (FAO, 1994), with an organic matter content 4.5%, CEC 12 meq 100 g<sup>-1</sup>  
148 and pH 6.0, was collected from a field close to the buffer strips at Balruddery farm. This soil was air-  
149 dried and sieved and then was either left non-fertilised (Control), or amended with a range of sources  
150 of chopped green manure, combinations of the green manure and chemical fertiliser (integrated  
151 nutrient management [INM]) or chemical fertiliser alone, detailed in supplemental Table S2. All  
152 treatments were replicated six times. The nutrient addition level was based on the rate of addition of P  
153 to achieve sufficient P for the cultivar of barley used in this experiment on this soil and was derived  
154 from previous studies (George et al., 2011). Specifically, the nutrient treatments were designed to be  
155 equivalent to the input of 20 t (fresh weight) green manure per hectare of soil to 25 cm depth (3.0 x  
156 10<sup>6</sup> kg soil) and were approximately equivalent to the addition of 50 kg P ha<sup>-1</sup> and 250 kg N ha<sup>-1</sup> when  
157 applied as a mixed green manure at the 100% dose rate. This provides a level of P adequate for plant  
158 growth, but due to the natural P:N ratio of the material provides a high rate of N fertilisation. All  
159 chopped biomass, ground fertiliser granules (NPK 22-4-14) and chemicals (NH<sub>4</sub>NO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub>),  
160 were mixed thoroughly with soil in a cement mixer for 15 minutes (75 rpm). Control soils were given  
161 the same mixing treatment without any soil amendments. Filter papers (90 mm diameter - Whatman  
162 International Ltd, Maidstone, UK), were placed in the bottom of 1 L (13 cm diameter, 11 cm depth)  
163 plastic pots to prevent soil loss and the pots were filled with 1 kg of the variously prepared soils. The  
164 pots were watered to 80% field capacity (equivalent to 40-50 kPa water potential) as determined by  
165 gravimetric water content and maintained at this level by daily watering to weight with deionised  
166 water. Pots were incubated in the glasshouse for 28 days prior to planting.

#### 167 Plant growth

168 Seeds from a commonly grown local barley variety (*Hordeum vulgare* cv. Optic) were germinated in  
169 90 mm diameter Petri dishes containing 0.5% distilled water agar [1 g of BDH Agar (VWR, UK) per  
170 200 ml distilled water]. At four days post germination, seedlings were planted (one per pot) in the pots



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

#### 181 Plant tissue analysis

182 Number of tillers, above-ground biomass (biomass), number of heads, head dry weight, and grain  
183 weight (yield) were recorded. The number of tillers for each replicate was counted before the  
184 harvested plant material was oven-dried at 70 °C for 4 days after which plant dry weight and head dry  
185 weight were recorded. Seeds were detached from the heads, cleaned with the aid of an Agriculex CB-  
186 1: Column Blower with an acrylic trash catcher (Agriculex, Guelph, Canada) and weighed.

187 The frozen flag leaf samples were freeze dried for 48 hours using a Sciquip CHRIST Alpha 1-2 freeze  
188 dryer (SciQuip, Shropshire, UK) and then ground using a QIAGEN TissueLyser II (QIAGEN, Hilden,  
189 Germany). Nitrogen and C concentrations in plant tissues were determined by the combustion (plant  
190 flour of 2-5 mg) in pure oxygen under static conditions using a CE440 Elemental Analyser (Leeman  
191 Labs, NH, USA). Tissue P concentrations were determined on the powdered leaf material (50 mg)  
192 which was digested in 15.8 M nitric acid HNO<sub>3</sub> (Aristar grade, VWR International, Poole, UK) for 20  
193 min at 180°C and thereafter oxidised in 1 ml of 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in closed vessels  
194 using a MARSXpress microwave oven (CEM, Buckingham, UK). After digestion, the solution was  
195 made up to a final volume of 50 mL with dH<sub>2</sub>O. Phosphate was determined using Malachite Green  
196 reagent (Irving and Mclaughlin, 1990) using a Multiskan GO UV-Vis spectrophotometer (Thermo  
197 Scientific, MA, USA) at 620 nm. Nutrient accumulation in above ground tissue of barley plants and  
198 individual bufferstrip species was calculated by multiplying the shoot biomass with the nutrient

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

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

202 | A selection of 33 species of annual and perennial plants which can be found present in Scottish buffer  
203 | strips, with growth habits that were considered useful for green manure production and were available  
204 | to purchase as seed from merchants, were grown in pots containing a sand/compost mix under high  
205 | P and N input conditions. Seeds of the individual species were pre-germinated in petri dishes  
206 | containing 0.5% distilled water agar [1 g of BDH Agar (VWR, UK) per 200 ml distilled water] then  
207 | transferred to 1 kg pots (one plant per pot) containing 50:50 sand, compost mix. Five replicates of  
208 | each species were planted. Pots were watered daily with 30 ml of a solution containing 32 mg P L<sup>-1</sup>  
209 | and 320 mg NO<sub>3</sub> L<sup>-1</sup>, a dose considered to be equivalent to the influx of nutrients to a buffer strip  
210 | under nutrient excess conditions (Stutter, personal communication). All other nutrients were supplied  
211 | weekly at the following concentrations 2 mM KNO<sub>3</sub>, 1 mM MgSO<sub>4</sub>, 80 μM FeEDTA, and  
212 | micronutrients [30 nM H<sub>3</sub>BO<sub>3</sub>, 6 μM CuSO<sub>4</sub>, 6 μM MnSO<sub>4</sub>, 0.6 μM ZnSO<sub>4</sub>, 42 nM NH<sub>4</sub>Mo<sub>7</sub>, 12 μM  
213 | Co<sub>4</sub>(NO<sub>3</sub>)<sub>2</sub>] at a rate of 25 mL per pot per week. The newest fully expanded leaf was sampled just  
214 | prior to harvest, which occurred at the onset of flowering, and stored at -20°C for shoot P analysis.  
215 | Tissue P concentrations were determined as described in Experiment 1. Plants were then harvested  
216 | at flowering stage by cutting at the pot surface and the above ground plant material was oven dried at  
217 | 70°C for 4 days. Dried plants were then weighed and the above ground biomass recorded.  
218 | Phosphorus accumulation (mg P plant<sup>-1</sup>) was calculated as the product of biomass and P  
219 | concentration in the shoot tissue.

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

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

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

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

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

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

254 Soil P was determined using the sodium bicarbonate (NaHCO<sub>3</sub>) extraction method (Olsen et al.,  
255 1954). In 50 mL centrifuge tubes, 0.5 g of soil and 5 mL of 0.5M NaHCO<sub>3</sub> were shaken for 60 minutes.  
256 The tubes were then centrifuged for 5 minutes at 5100 rpm. After centrifugation the supernatant was

257 stored at 4°C until analysis. Phosphate was determined using Malachite Green reagent, described in  
258 Experiment 1. Ammonia-N, NO<sub>3</sub>-N, and total organic carbon (TOC) were measured using the  
259 potassium chloride (KCl) extraction method. To prepare the extract, 5 g of soil and 25 ml of 1 M KCl  
260 were placed in 50 mL centrifuge tubes and tubes were shaken (45 rpm) for 45 minutes and then  
261 centrifuged for 5 minutes at 5100 rpm. The supernatant was stored at 4°C until analysis. Ammonia-N  
262 and NO<sub>3</sub>-N were determined spectrophotometrically using a Konelab Aqua 20, Discrete Analyser,  
263 (Thermo Electron Corporation, Finland). Total organic carbon was determined using an OI Analytical  
264 1010 analyser (OI Analytical, TX, USA).

#### 265 *Statistical analysis*

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

## 274 **Results**

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

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

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

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

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

#### 299 *Screen of riparian buffer strip species (Experiment 2)*

300 The selection of 33 typical riparian buffer strip species demonstrated a large significant ( $P<0.001$ )  
301 variation in biomass response (90-fold) and P accumulation (38-fold) when treated with excess inputs  
302 of N and P (Figure 2). Biomass (above ground dry weight) ranged from 0.09 g for *Juncus effusus* to  
303 41.58 g for *Holcus mollis*. P accumulation ranged from 1.38 mg P plant<sup>-1</sup> for *Cardamine pratenses* to  
304 52.73 mg P plant<sup>-1</sup> for *Agrostis canina*. The species order for P accumulation was not correlated  
305 (N.S.) with that for biomass.

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

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

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

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

317 Nutrient concentrations of the soils following the addition and incubation of single species green  
318 manures show some significant ( $P<0.001$ ) differences for NH<sub>4</sub>-N, NO<sub>3</sub>-N and TOC (Table 3).  
319 Ammonium was greater in soils amended with green manure from *Festuca rubra*, *Trifolium repens*,  
320 *Lolium perenne* and the 4 species mixed compared to the control, the NPK fertiliser and *Myosotis*  
321 *arvensis* treatments. For NO<sub>3</sub>-N, the NPK and *Trifolium repens* treatments were significantly  
322 ( $P<0.001$ ) greater than all other treatments at 33.39 mg kg<sup>-1</sup> and 31.435 mg kg<sup>-1</sup>, respectively. Total  
323 Organic Carbon was significantly ( $P<0.001$ ) greater for the *Lolium perenne* and 4 species mix than all  
324 the other treatments. P concentrations however, were not significantly affected by the various nutrient  
325 sources.

## 326 Discussion

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

341 [differences in species used in green manure systems in the tropics compared to temperate regions,](#)  
342 [with a greater propensity of legume species used in the former leading to greatly improved N nutrition](#)  
343 [in tropical green manure systems.](#)

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

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

370 that species with large biomass and nutrient accumulation (e.g. *Argrostis canina*) would be optimal for  
371 green manure systems, compared to those with small biomass and nutrient accumulation (e.g.  
372 *Myosotis arvensis*). Use of these selected species would go some way to mitigating the impact of  
373 nutrient accumulation in buffer strips and reduce the potential of these to act as a source of polluting  
374 nutrients (Borin et al. 2005). Based on observations made in this study, we are able to **calculate**  
375 **estimate** that the species accumulating the largest amounts of P would be able to sequester around  
376 10% of all the P accumulating in a **typical** buffer strip **of eastern Scotland** in a given year, while the  
377 poorest accumulator would sequester less the 1%.

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

388 The addition of green manure amendments from the specific species had no impact on the P  
389 availability in the soil, but neither did the addition of NPK (Table 3). In contrast, the soil  $\text{NH}_4$  levels  
390 were increased by all species specific organic amendments except the *Myosotis arvensis* and when  
391 all species were added together (Table 3), while,  $\text{NO}_3$  was only increased in the soil with the addition  
392 of NPK and the *Trifolium repens* green manure. In the case of P, none of the amendments had the  
393 predicted effect on the availability of P in the soil expected due to their C:P ratio. It was clear that the  
394 addition of the leguminous green manure had a large impact on the availability of N increasing both  
395  $\text{NH}_4$  and  $\text{NO}_3$ . This result was also seen by a number of other authors (Diekman et al., 1993; Cobo et  
396 al., 2002a) and has been attributed to enhanced microbial activity in soils amended with legume  
397 biomass (Elfstrand et al., 2007). The larger C:N of the other green manure amendments may explain  
398 their limited impact on  $\text{NO}_3$  availability (Cobo et al., 2002b) and all but *Myosotis arvensis* enhanced



399  $\text{NH}_4$  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 mineralisation 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.

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

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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.

574

575

	Tillers (n)	Heads (n)	Head DW g pot <sup>-1</sup>	P con mg g <sup>-1</sup>	P acc mg plant <sup>-1</sup>	C con mg g <sup>-1</sup>	C acc mg plant <sup>-1</sup>	N con mg g <sup>-1</sup>	N acc mg plant <sup>-1</sup>
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

576

577



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

581

582

583 Table 3: Available P, NH<sub>4</sub> and NO<sub>3</sub> 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

<b>Nutrient source</b>	<b>P (mg kg<sup>-1</sup>)</b>	<b>NH<sub>4</sub>-N (mg kg<sup>-1</sup>)</b>	<b>NO<sub>3</sub>-N (mg kg<sup>-1</sup>)</b>	<b>TOC (mg kg<sup>-1</sup>)</b>
Control	28.4	0.4	18.1	20.7
NPK	28.4	0.4	33.3	20.9
<i>Myosotis arvensis</i>	28.7	0.4	14.1	23.1
<i>Festuca rubra</i>	27.9	0.7	14.2	24.5
<i>Trifolium repens</i>	29.4	0.8	31.4	26.5
<i>Lolium perenne</i>	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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592 **Figure Legends**

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

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

608 Figure 3: Yield of barley after growth in soil amended with green manure from specific individual  
609 species typical of riparian buffer strips in Scotland and the same sources of green manure when  
610 applied in an integrated nutrient management (INM) system where half of the required nutrient is  
611 provided as inorganic sources (Experiment 3). All soils received the equivalent of 50 kg P ha<sup>-1</sup> and  
612 ~~250 between 100 and 500~~ kg N ha<sup>-1</sup> (See Table S2), ~~dependent on the N:P ratio of the material~~  
613 ~~added~~. Plants were grown for 14 weeks in controlled conditions and compared to an unfertilised  
614 control. The data are the mean of six replicates and the error bars represent +/- one standard error of  
615 the mean. No LSD is presented as there were no significant differences observed.

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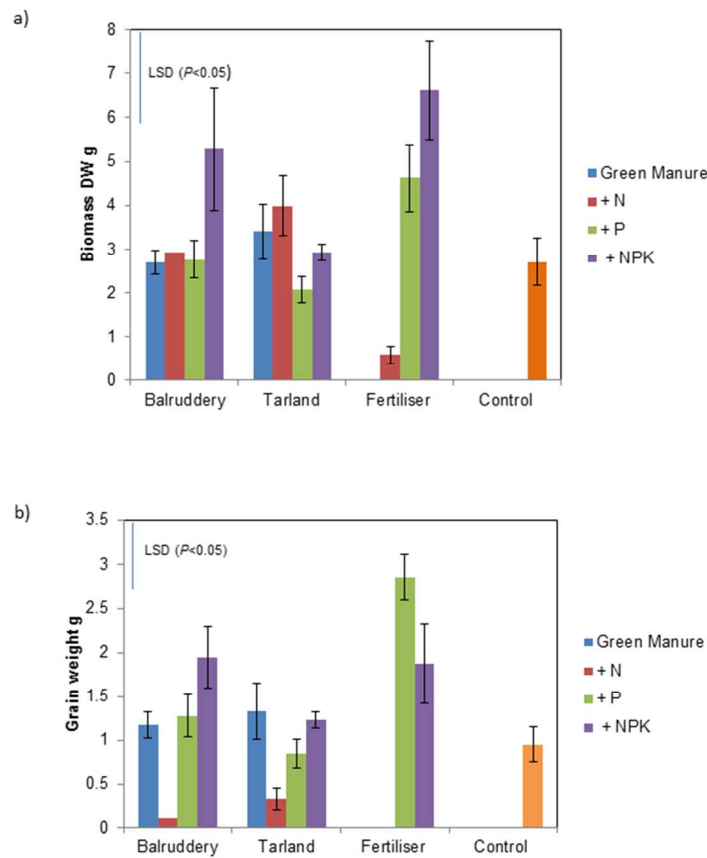


Figure 1: a) Biomass production and b) yield of barley grown in soils amended with green manure and green manure integrated with nitrogen (+N), phosphorus (+P) and fertiliser (+NPK) compared to a NPK (fertiliser) and no addition control. Green manures were sourced from existing conventionally managed 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.

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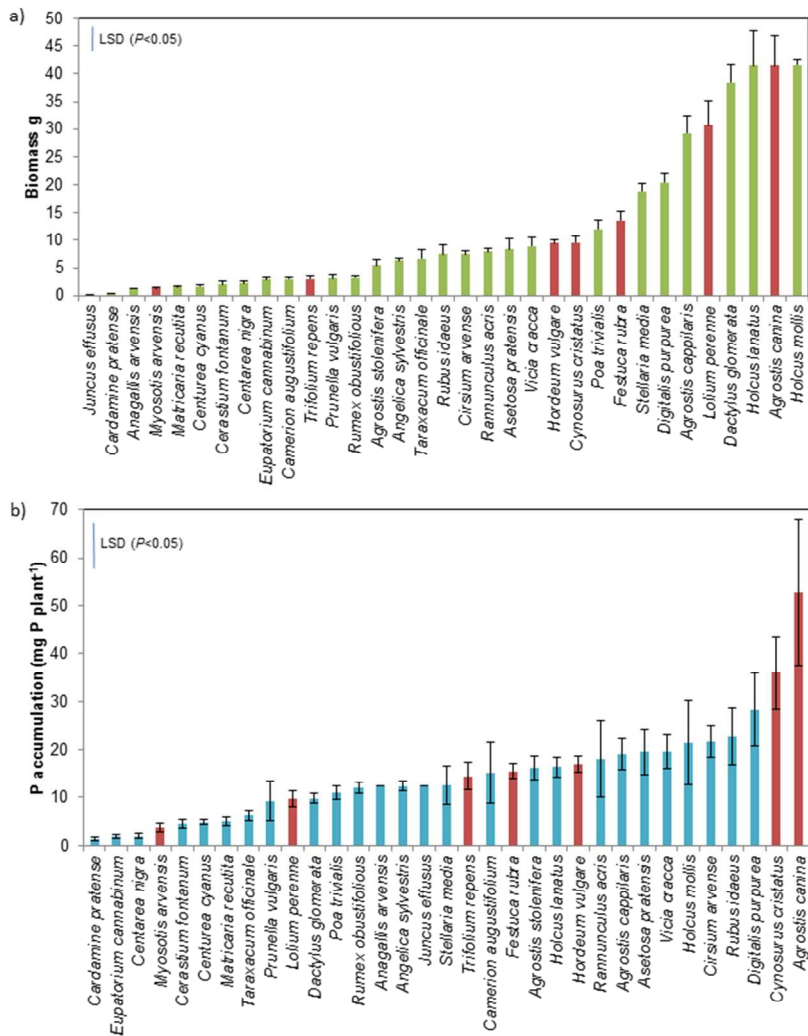


Figure 2: a) Biomass production and b) P accumulation of a range of common buffer strip species grown for 12 weeks in a sand compost mix amended with N and P to levels typical of a riparian buffer strip, such that these nutrients are in excess of requirement. Red bars represent the species which were 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.

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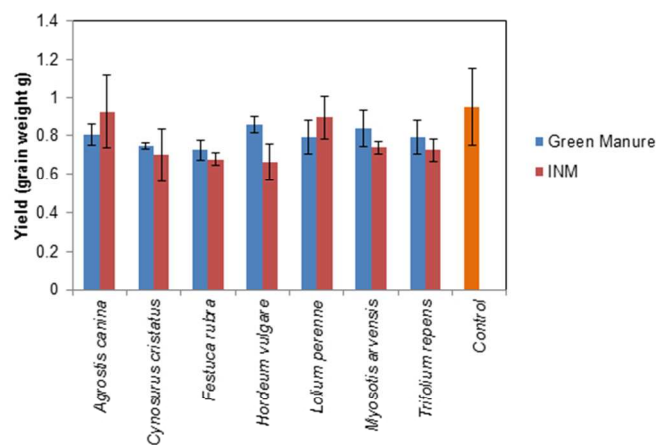


Figure 3: Yield of barley after growth in soil amended with green manure from specific individual species typical of riparian buffer strips in Scotland and the same sources of green manure when applied in an integrated nutrient management (INM) system where half of the required nutrient is provided as inorganic sources (Experiment 3). All soils received the equivalent of 50 kg P ha<sup>-1</sup> and between 100 and 500 kg N ha<sup>-1</sup> (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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## 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	<i>Aegopodium podagraria</i> <i>Anthriscus sylvestris</i> <i>Arrhenatherum elatius</i> <i>Chamaenerion angustifolium</i> <i>Cirsium arvense</i> <i>Crataegus monogyna</i> <i>Dactylis glomerata</i> <i>Elytrigia repens</i> <i>Epilobium angustifolium</i> <i>Equisetum sp</i> <i>Filipendula ulmaria</i> <i>Fumaria sp</i> <i>Galium aparine</i> <i>Heracleum sphondylium</i> <i>Lolium perenne</i> <i>Matricaria discoidea</i> <i>Matricaria recutita</i> <i>Myosotis arvensis</i> <i>Plantago major</i> <i>Poa annua</i> <i>Poa trivialis</i> <i>Polygonum aviculare</i> <i>Rubus fruticosus</i> <i>Rubus idaeus</i> <i>Rumex sp</i> <i>Senecio vulgaris</i> <i>Taraxacum officinalis</i> <i>Trifolium repens</i> <i>Tripleurospermum inodorum</i> <i>Urtica dioica</i>	<i>Agrostis stolonifera</i> <i>Dactylis glomerata</i> <i>Festuca rubra</i> <i>Holcus lanatus</i> <i>Holcus mollis</i> <i>Juncus effusus</i> <i>Phragmites australis</i> <i>Poa trivialis</i> <i>Anthriscus sylvestris</i> <i>Cirsium arvense</i> <i>Filipendula ulmaria</i> <i>Galium aparine</i> <i>Ranunculus repens</i> <i>Rumex obtusifolios</i> <i>Urtica dioica</i>	<i>Agrostis canina</i> <i>Agrostis cappilaris</i> <i>Agrostis stolonifera</i> <i>Anagallis arvensis</i> <i>Angelica sylvestris</i> <i>Asetosa pratensis</i> <i>Camerion angustifolium</i> <i>Cardamine pratense</i> <i>Centarea nigra</i> <i>Centurea cyanus</i> <i>Cerastium fontanum</i> <i>Cirsium arvense</i> <i>Cynosurus cristatus</i> <i>Dactylus glomerata</i> <i>Digitalis purpurea</i> <i>Eupatorium cannabinum</i> <i>Festuca rubra</i> <i>Holcus lanatus</i> <i>Holcus mollis</i> <i>Hordeum vulgare</i> <i>Juncus effusus</i> <i>Lolium perenne</i> <i>Matricaria recutita</i> <i>Myosotis arvensis</i> <i>Poa trivialis</i> <i>Prunella vulgaris</i> <i>Ranunculus acris</i> <i>Rubus idaeus</i> <i>Rumex obtusifolios</i> <i>Stellaria media</i> <i>Taraxacum officinale</i> <i>Trifolium repens</i> <i>Vicia cracca</i>
Orders	Apiales (2) Aquifoliales (1) Asparagales (1) Asterales (14) Boraginales (3) Brassicales (1) Caryophyllales (6) Dennstaediales (1) Dipsacales (1) Ericales (1) Fabales (3) Fagales (3) Gentianales (1) Lamiales (6) Malpighiales (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) Dennstaediales (1) Dipsacales (1) Ericales (1) Fabales (5) Gentianales (2) Lamiales (11) Malpighiales (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.

Soil Treatment	Nutrient Addition (% dose)			
	N	P	NPK	Green Manure (20t ha <sup>-1</sup> )
(100 % dose rate)	(250 kg N ha <sup>-1</sup> )	(50 kg ha <sup>-1</sup> )	(250 kg N ha <sup>-1</sup> ; 50 kg P ha <sup>-1</sup> )	(250 kg N ha <sup>-1</sup> ; 50 kg P ha <sup>-1</sup> )
Control	-	-	-	-
Balrulderry Green Manure	-	-	-	100
Tarland Green Manure	-	-	-	100
Balrulderry Green Manure +N	50	-	-	50
Tarland Green Manure +N	50	-	-	50
Balrulderry Green Manure + P	-	50	-	50
Tarland Green Manure +P	-	50	-	50
Balrulderry Green Manure +NPK	-	-	50	50
Tarland Green Manure +NPK	-	-	50	50
<i>A. canina</i> Green Manure	-	-	-	100
<i>C. cristatus</i> Green Manure	-	-	-	100
<i>F. rubra</i> Green Manure	-	-	-	100
<i>H. vulgare</i> Green Manure	-	-	-	100
<i>L. perenne</i> Green Manure	-	-	-	100
<i>M. arvensis</i> Green Manure	-	-	-	100
<i>T. repens</i> Green Manure	-	-	-	100
<i>A. canina</i> Green Manure +NPK	-	-	50	50
<i>C. cristatus</i> Green Manure +NPK	-	-	50	50
<i>F. rubra</i> Green Manure +NPK	-	-	50	50
<i>H. vulgare</i> Green Manure +NPK	-	-	50	50
<i>L. perenne</i> Green Manure +NPK	-	-	50	50
<i>M. arvensis</i> Green Manure +NPK	-	-	50	50
<i>T. repens</i> 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<sup>-1</sup> of soil to 25cm depth and were on average the equivalent of the addition of 50kg P ha<sup>-1</sup> and 250kg N ha<sup>-1</sup> when applied at the 100% dose rate.