AGRICULTURE AND FOOD DEVELOPMENT AUTHORITY

TITLE: Nitrogen yield advantage from grass-legume mixtures is robust over a wide range of legume proportions and environmental conditions

AUTHORS: Suter, M., Connolly, J., Finn, J.A., Loges, R., Kirwan, L., Sebastià, M.T., Lüscher, A.

1 **Nitrogen yield advantage from grass-legume mixtures is robust over a wide range of**

2 **legume proportions and environmental conditions**

3

4 Running head: Nitrogen yield from grass-legume mixtures

5

6   Suter, M.[1], Connolly, J.[2], Finn, J.A.[3], Loges, R.[4], Kirwan, L.[5], Sebastià, M.T.[6,7], Lüscher, A.[1]

7

8   [1]Agroscope, Institute for Sustainability Sciences ISS, Reckenholzstrasse, Zürich, Switzerland

9 [2]School of Mathematical Sciences, University College Dublin, Dublin 4, Ireland

10 [3]Teagasc, Environment Research Centre, Johnstown Castle, Wexford, Ireland

11   [4]Institut für Pflanzenbau und Pflanzenzüchtung, Christian-Albrechts-Universität, Kiel,

12   Germany

13 [5]Waterford Institute of Technology, Cork Road, Waterford, Ireland

14 [6]Forest Sciences Centre of Catalonia, Solsona, Spain

15 [7]Dept HBJ, ETSEA, Universitat de Lleida, Lleida, Spain

16

17 Corresponding author: Matthias Suter, phone: +41 58 468 75 90,

18   fax: +41 58 468 72 01, email: matthias.suter@agroscope.admin.ch

19

23

24 Type of paper: Primary research article

Abstract

26   Current challenges to global food security require sustainable intensification of agriculture

27   through initiatives that include more efficient use of nitrogen (N), increased protein self-

28 sufficiency through home-grown crops, and reduced N losses to the environment. Such

29   challenges were addressed in a continental-scale field experiment conducted over three years,

30 in which the amount of total nitrogen yield $_{(Ntot)}$ and the gain of N yield in mixtures as

31   compared to grass monocultures $_{(Ngainmix)}$ was quantified from four-species grass-legume

32 stands with greatly varying legume proportions. Stands consisted of monocultures and

33 mixtures of two N_2 fixing legumes and two non-fixing grasses.

34 The amount of $_{Ntot}$ of mixtures was significantly greater ($P \leq 0.05$) than that of grass

35   monocultures at the majority of evaluated sites in all three years. N_{tot} and thus $N_{gainmix}$

36 increased with increasing legume proportion up to one third of legumes. With higher legume

37   percentages, $_{Ntot}$ ^{and} $_{Ngainmix}$ did not continue to increase. Thus, across sites and years,

38 mixtures with one third proportion of legumes attained ~95% of the maximum $_{Ntot}$ acquired

39 by any stand and had 57% higher $_{Ntot}$ than grass monocultures.

40 Realized legume proportion in stands and the relative N gain in mixture $_{(Ngainmix/Ntot}$ in

41 mixture) were most severely impaired by minimum site temperature ($R = 0.70$, $P = 0.003$ for

42   legume proportion; $R = 0.64$, $P = 0.010$ for $_{Ngainmix/Ntot}$ in mixture). Nevertheless, the relative

43   N gain in mixture was not correlated to site productivity ($P = 0.5\ 00$), suggesting that, within

44 climatic restrictions, balanced grass-legume mixtures can benefit from comparable relative

45   gains in N yield across largely differing productivity levels.

46 We conclude that the use of grass-legume mixtures can substantially contribute to resource-

47   efficient agricultural grassland systems over a wide range of productivity levels, implying

48 important savings in N fertilizers and thus greenhouse gas emissions and a considerable

49 potential for climate change mitigation.

50 Introduction

51   Global food security is currently challenged by the increasing demands for food, including

52 meat and milk, which arise through the continuing growth of the world's population and

53   consumption (Godfray *et al.*, 2010; Smith & Gregory, 2013). At the same time, food

54 production is significantly affected by competition between food, feed and bioenergy,

55 demands from other economic sectors for land and water, and the need to maintain and

56 preserve ecosystem services and biodiversity (Thornton, 2010). Moreover, current food

57 production is highly nitrogen (N) limited (Cassman *et al.*, 2002), while the provision of

58   industrial N is largely based on fossil energy and its multiple impacts on the environment

59   (Galloway *et al.*, 2008; Davidson, 2009; Canfield *et al.*, 2010). Under a business-as-usual

60 scenario, it must be assumed that any increase in food provision will further aggravate the

61 pressure regarding the divergent demands for food security and environmental integrity

62   (Tilman *et al.*, 2002; Foley *et al.*, 2005). This challenge has recently reinforced the need for

63   more sustainable agriculture and sustainable intensification (Godfray *et al.*, 2010; Foley *et al.*,

64   2011).

65

66   Sustainable intensification of agriculture aims to raise productivity while at the same time

67   reduce its environmental impacts (Godfray *et al.*, 2010; Foley *et al.*, 2011; Lüscher *et al.*,

68   2014; Taube *et al.*, 2014). In the face of the prevailing N limitation, rising costs of inorganic

69   N fertilizers, and deleterious side-effects of excessive N application (Galloway *et al.*, 2008;

70   Canfield *et al.*, 2010), increased sustainability and improved N self-sufficiency can be gained

71   through home-grown N_2 fixing crops. Currently, grassland-based livestock production of

72 medium to high management intensity depends largely on high-yielding pure grass stands

73   requiring large inputs of mineral N fertilizers. Production and distribution of mineral N

74   fertilizers need large amounts of energy (Kitani *et al.*, 1999) and their application can result in

76 environment (Schils *et al.*, 2013; Schmeer *et al.*, 2014). For example, each kg of N produced

77 as ammonium nitrate in the industrial Haber-Bosch process consumes 58 MJ of energy and

78   emits 8.6 kg CO_2 equivalents (Kitani *et al.*, 1999; Ecoinvent Centre, 2010). Moreover,

79 according to the guidelines of the IPCC (2006), for every 100 kg of N fertilizer added to the

80 soil, on average 1.0 kg of N is emitted as N_2O, a GHG that is approximately 300 times more

81 potent than CO_2. At the same time, European livestock systems depend strongly on protein

82 imported from overseas. A major challenge, therefore, is to increase home-grown forage

83 protein with reduced input of mineral N fertilizers and at the same time reduce N losses to the

84   environment (Peyraud *et al.*, 2009; Lüscher *et al.*, 2014; Taube *et al.*, 2014).

85

86 Grass-legume mixtures offer the benefit of symbiotic N_2 fixation by legumes, which are able

87 to utilize atmospheric N_2 for their requirements and thereby produce more protein with less N

88 input. The amount of symbiotic N_2 fixation by legumes can be substantial and ranges from

89   100 to 380 kg ha^{-1} $year^{-1}$ in northern temperate/boreal regions (Ledgard & Steele, 1992;

90 Carlsson & Huss-Danell, 2003). Grown in mixtures with grasses, legumes meet their own N

91   demand by deriving more than 80% from symbiosis (Boller & Nösberger, 1987; Zanetti *et al.*,

92   1997; Nyfeler *et al.*, 2011) and consequently, the relative availability of soil N increases for

93   grasses ("N sparing", Temperton *et al.*, 2007). Besides symbiotic N_2 fixation, other processes

94 have been found to increase yield and efficiency in resource uptake by grass-legume mixtures.

95   These include facilitation, that is, N transfer from legumes to grasses (Høgh-Jensen &

96   Schjoerring, 1997; Pirhofer-Walzl *et al.*, 2012; Rasmussen *et al.*, 2013) and increased

97   exploitation of soil resources through spatial (deep- and shallow-rooting) or temporal niche

98   complementarity in resource uptake (van Ruijven & Berendse, 2005; Mueller *et al.*, 2013).

99 All of these processes can lead to considerable gains in N yield of mixtures compared to grass

100 monocultures; consequently, the use of such mixtures in agricultural grassland systems could

101   allow substantial reductions in the application of industrial N fertilizers.

102

103 Inclusion of legumes in grassland management has been proposed as an important strategy for

104   climate change mitigation in the agricultural sector (Smith *et al.*, 2008; Smith & Gregory,

105   2013; Bustamante *et al.*, 2014). The use of grass-legume mixtures in temporary grassland is

106 widely applicable and does not necessarily compromise harvest yield, thereby improving

107   agricultural efficiency (Kirwan *et al.*, 2007; Finn *et al.*, 2013). Moreover, the application is

108 practical and robust, as demonstrated over many years by the "Swiss Standard Mixtures" that

109 use up to eight species of grasses and legumes in temporary grassland to improve legume

110   persistence under various pedo-climatic conditions (Frey, 1955; Suter *et al.*, 2012). Grass-

111   legume mixtures offer also a sustainable farm management practice: there is no evidence of

112 significant emissions of N_2O arising from the process of symbiotic N_2 fixation (Rochette &

113   Janzen, 2005; Barton *et al.*, 2011) and thus, N_2 fixation has been removed as a direct source

114 of N_2O in the revised GHG guidelines of the IPCC (2006). Finally, mixing grasses with

115 legumes is a mitigation measure that can be implemented in the near future, which is an

116   important feature for meeting emission reduction targets (Smith *et al.*, 2013; Bustamante *et*

117   *al.*, 2014).

118

119 Although amounts of total N yield and N from symbiotic sources have been quantified in

120 temperate grassland (Ledgard & Steele, 1992; Carlsson & Huss-Danell, 2003), data from

121   arctic or continental ecosystems are rare. In such environments, low winter temperatures

122 and/or precipitation may hamper the legumes' growth, and accordingly, the benefit of

123   including legumes in mixtures to achieve high gains in N yield may be limited. Absolute

124 amounts of N from symbiosis seem to decrease at higher latitudes of Europe (Nesheim &

125 Oyen, 1994); however, there is no simple correlation between latitude and the N performance

126 of legumes (Carlsson & Huss-Danell, 2003). Any large-scale geographic influence acts in

127   concert with local pedo-climatic conditions that also interact with the type and intensity of

128 management, all of which may strongly affect the legumes' growth.

129

130 The COST Action 852 entitled "Quality legume-based forage systems for contrasting

131   environments" (www.cost.eu/domains_actions/fa/Actions/852) aimed at increasing the

132 quantity and quality of home-grown protein from regionally adapted legume-based forage

133   systems. To this aim, a coordinated continental-scale field experiment was established, the

134 "Agrodiversity Experiment", focusing on intensively managed, mown temporary grassland as

135 a model system. Monocultures and mixtures comprised of two grasses (non N_2 fixing) and

136 two legumes (N_2 fixing), and were set up to result in a broad range of legume proportion in

137   stands (Kirwan *et al.*, 2007). Here, we report on the benefits of legumes to total N production

138   in these grass, legume, and mixed swards by analyzing the total N yield $_{(Ntot)}$ and realized

139 legume proportion of swards, and the potential N yield gain in mixtures as compared to grass

140   monocultures $_{(Ngainmix)}$. To our knowledge, this is the first study to directly relate N yields in

141   mixtures and monocultures to greatly differing stand legume proportions across largely

142   contrasting pedo-climatic conditions, which will reveal the potential of these grassland

143   systems for resource-efficient, sustainable agriculture through savings in N fertilizers and thus

144 GHG emissions. The following specific questions were addressed:

145   1. Is $_{Ntot}$ consistently higher in grass-legume mixtures as compared to grass monocultures?

146   2. Is $_{Ntot}$ ^{and} $_{Ngainmix}$ affected by legume proportion in the sward? If yes, how much does the

147   effect of legume proportion on $_{Ntot}$ ^{and} $_{Ngainmix}$ vary across sites?

148   3. Is the effect of legume proportion on $_{Ntot}$ ^{and} $_{Ngainmix}$ persistent over three years? Does

149   legume proportion itself persist over time?

150   4. Are realized legume proportion, N_{tot} in mixture, and the relative N gain in mixture

151   ($N_{gainmix}/N_{tot}$ in mixture) related to climatic variables and to productivity levels across

152   sites?

153 **Material and Methods**

154 *Experimental design*

155 At each of sixteen sites and following a common protocol, four monocultures and eleven

156   mixtures were established at two levels of seed density for a total of 30 stands per site. The

157   sites spanned a gradient of climate from Atlantic to continental and from temperate to arctic

158   (Fig. 1, Table S1, Supporting Information). Regarding climate and productivity, mean annual

159 temperature of the sixteen sites ranged between 4.2 and 10.9 °C, precipitation between 492

160 and 1556 mm year^{-1}, and productivity of aboveground biomass between 2 and 14 t DM ha^{-1}

161   year^{-1} (see below). Full information to all sites is given in Kirwan *et al.* (2014), where

162   geographic coordinates and further details on climate and soils are provided. The selection of

163   species used for experimentation focused on i) species known to achieve high forage quantity

164 and quality in systems of intensive grassland management, and ii) functional types of species

165 that were expected to maximise complementarity in resource use. Four functional types of

166   species were initially defined as the factorial combination of traits being associated with the

167 manner of N acquisition (non N_2 fixing grasses *vs.* N_2 fixing legumes), and temporal pattern

168   of species' growth (fast establishment *vs.* temporally persistent), resulting in the following set:

169   fast establishing grass (G1), temporally persistent grass (G2), fast establishing legume (L1),

170 temporally persistent legume (L2). These four functional types were consistent across all

171   sixteen sites although the species selected at sites varied depending on geographical region

172   (Table 1).

173

174 The four monocultures consisted of one of each of G1, G2, L1, or L2, and mixtures contained

175   all four functional types of species in varying relative abundances following a simplex design

176 (Cornell, 2002). This was achieved by systematically varying the sown species proportions in

177 mixtures (Table S2, Supporting Information) to result in four mixtures dominated in turn by

178   one species (70% of one species, 10% of the three others), six mixtures co-dominated in turn

179 by pairs of species (40% of each of two species, 10% of the two others), and one mixture with

180 equal species proportions (25% of each species). Doing so, sown legume proportions across

181   different communities were 0, 20, 50, 80, and 100% (see also Kirwan *et al.*, 2007 for further

182   details on the design).

183

184 *Maintenance and measurements*

185 Plots were fertilized with commercial N fertilizer. The amount was constant for all plots and

186   for all years of experimental duration within individual sites, but varied among sites to range

187 from 0 to 150 kg N ha^{-1} year^{-1} (Table S1, Supporting Information). This range of N

188   application reflected background productivity levels across the large climatic gradient as well

189   as variations in types and fertility of soils. Note that the maximum level of 150 kg N ha^{-1} year^{-}

190    $_1$ is far below highest levels of N application to grass monocultures in intensive grassland

191   management and that N application of up to 150 kg ha^{-1} year^{-1} did not impair positive grass-

192   legume interactions at one of the study sites (Nyfeler *et al.*, 2009). Background levels of

193 phosphorus and potassium in experimental plots were adjusted to non-limiting amounts

194 (Kirwan *et al.*, 2014). Aboveground biomass of plots was harvested several times per year

195   following the agronomic practice at each site (Table S1, Supporting Information), and

196 representative subsamples of harvested yield were sorted into the four sown and pooled

197 unsown species. Drying to constant weight and summing over harvests allowed computing of

198   the total harvested dry mass per year and species' fractional contributions. Importantly,

199 measurements were only recorded in the first three full years of production in this temporary

200 grassland; thus, the sowing year was not considered. This restriction was imposed to evaluate

201   the fully established system.

202

203 *Record of climatic data*

204 Data on climatic conditions were monitored by weather stations at each of the sixteen sites.

205 Daily precipitation was summed and mean daily temperature was averaged across all days of

206 the year to give annual precipitation and mean annual temperature per site. Moreover, daily

207 minimum and maximum temperatures were used to compute the annual minima and maxima

208 per site as the average of the ten days with most extreme values. This was of specific interest

209 because extreme temperatures are assumed to especially impair legumes' growth and N_2

210 fixation (Lynch & Smith, 1993; Zhang *et al.*, 1995).

211

212 *Analysis of N concentration in plant material*

213 Nitrogen concentration $_{(Nconc)}$ was measured at each harvest from a representative subsample

214 of total harvested biomass from each plot, with biomass being dried to constant weight at 65

215 °C and ground to pass through a 1 mm sieve. The value of $_{Nconc}$ of all samples was

216 determined by near-infrared reflectance spectroscopy (NIRS) at one lab (Christian-Albrechts

217 University, Kiel, Germany) using a NIRSystems 5000 monochromator (FOSS, Silver Spring,

218 USA). See Appendix A, Supporting Information, for validation of the NIRS method. For a

219 subset of sites, $_{Nconc}$ was not measured at all three experimental years and from all plots of the

220 design (see Table S1, Supporting Information). Note that temporary grassland in crop rotation

221 systems is generally maintained for a sowing year and one or two subsequent production

222 years. Here, we measured $_{Nconc}$ for two production years for most sites (year 1 and 2), while

223 half the sites also had data for a third year (year 3), resulting in a total of 350, 304, and 167

224 analyzed plots in years 1, 2, and 3, respectively. Importantly, included sites covered the full

225 pedo-climatic range in all years. There were a total of 36 site-years of data.

226

227 *Data analyses*

228 We aimed to analyze the total annual N yield (N_{tot}) per plot. To achieve this, values of $_{Nconc}$ of

229 each harvest were first multiplied by total dry matter per harvest to receive the N yield per

230 harvest and plot. Annual N_{tot} per plot was then computed as the sum over all harvests and

231   reflects therefore an N output of the system that is seasonally weighted for variability in N_{conc}

232 and biomass yield.

233

234   *i) Comparisons of mixtures against grass monocultures*

235 Annual N_{tot} of mixtures was initially compared against N_{tot} of the average *grass* monoculture,

236 as this comparison reflected a test of mixed stands (all of which included legumes) against

237 stands sown only with grass species. Wilcoxon rank sum tests were applied for inference.

238

239   *ii) Testing the effect of legume proportion on total nitrogen yield*

240 The effect of legume proportion (PL_{eg}) the on annual N_{tot} was analyzed applying a regression

241   approach following Nyfeler *et al.* (2011), which used the following basic notation:

242 $N_{tot} = fl_0 + p_i P_{,,eg} + p_2 PL_{eg}^2 + p3 PL_{eg}^3 + aD + yDeltaBiomass + E$ eqn. 1

243 In this formulation, PL_{eg} denotes the summed proportions of the two legumes L1 and L2,

244 using the harvested biomass proportions of the previous year (sown proportions were used for

245 year 1) as predictor variables to overcome confounding effects of year-to-year changes in

246 community composition on N_{tot}. Thus, $_{Ni}$ to)63 estimate the linear and non-linear effects of

247   stand legume proportion on N_{tot}. The intercept, P_0, gives the estimate of N_{tot} if $PLeg = 0$, i.e.

248 for grass monocultures including potential weeds. The effect of seed density is estimated by

249 a, with D denoting the level of seed density coded as -1 and +1 for low and high density,

250 respectively, so all other terms are estimated at average density. The effect of fluctuations in

251   stand biomass (*DeltaBiomass*) on the response variable is estimated by the y coefficient (for

252 details on computation of

DeltaBiomass see Appendix A, Supporting Information). The error

253   ε is assumed normally distributed with zero mean and variance a^2.

254

255 Equation 1 was extended to a linear mixed model where all coefficients were estimated for

256 each of three years, and where random coefficients were added to estimate the general stand

257 performance while allowing for variation across sites for each of the three years (Pinheiro &

258   Bates, 2009) (see eqn. S1, Appendix A, Supporting Information, for detailed notation).

259 Inference on fixed main effects of the linear mixed model was based on single term deletion

260   from a main effects model as specified in eqn. 1 (each effect in turn, including $\sim\sim_{eg}^{2}$ and

261    $\sim\sim_{eg}^{3}$) and subsequent F-tests for comparison of models while applying the Kenward-Roger

262 method to determine the approximate denominator degrees of freedom (Kenward & Roger,

263   1997); interactions were similarly tested but from a model that included all effects. The range

264 of legume proportion for which $_{Ntot}$ was significantly different from its maximum was

265 computed using the Johnson Neyman technique (Johnson & Neyman, 1936) as applied in

266   Suter *et al.* (2007).

267

268   In regressions of eqs. 1 and S1 (Appendix A, Supporting Information), species G1, G2, L1,

269 and L2 are grouped into grasses and legumes according to their functional ability to fix N_2 (or

270 not), and such analysis does not include interactions between species regarding temporal

271   pattern of species' growth. This grouping was justifiable because preliminary analyses

272 revealed that interactions affecting $_{Ntot}$ were mainly between non-fixing grasses and N_2 fixing

273   legumes, but to a far lesser extent between fast establishing and temporally persistent species

274 within grasses and legumes (see Appendix A, Supporting Information, for details and

275   inference on pooling individual species performances).

276

13

277   *iii) Computing gain of N yield in mixture*

278 Total N yield in mixed grass-legume stands and the respective monocultures can be

279   subdivided into different parts to illustrate the role of legumes and their interactions with

280 grasses. In grass monocultures (legume proportion = 0), N_{tot} accumulated by the sward

281   derives solely from the soil and from fertilizer N (quantity A, dashed white line for reference,

    282 Fig. 2). In legume monocultures (legume proportion = 1), N_{tot} is often greater (A + B), due

283 mainly to symbiotic N_2 fixation of legumes. Mixing grasses with legumes should lead

  284 therefore at first to a linear increase of accumulated N by the stand with increasing mixture

  285 legume proportion through the contribution of the legume component, i.e. through a constant

  286 amount of N added per unit of legumes (continuous white line). In addition, positive grass-

287 legume interactions such as stimulation of the rate of symbiotic N_2 fixation (% N derived

288   from symbiosis in the legume) when grass is present (Nyfeler *et al.*, 2011), N transfer (Høgh-

289   Jensen & Schjoerring, 1997; Zanetti *et al.*, 1997) or increased utilization of fertilizer and soil

  290 N resources through temporal and/or spatial niche complementarity (Mueller *et al.*, 2013) can

291   result in a nonlinear surplus of N yield (C, Fig. 2), resulting in a total N yield of A+B+C in

292 mixed swards.

293

294 This study aimed to quantify the amount of N yield gain in mixtures as compared to grass

   295 monocultures. This was achieved by computing the difference between N_{tot} in mixture and

296 N_{tot} in grass monoculture using the estimates of the regression model (based on eqn. S1,

  297 Appendix A, Supporting Information). This quantity of N yield gain in mixture is hereafter

    298 termed $N_{gainmix}$ (= N_{tot} in mixture minus N_{tot} in grass monoculture, quantity B + C in Fig. 2),

 299 and was calculated both for the mean across all sites (using the fixed parameter estimates of

300 the regression) and for each of the sixteen individual sites (using the variation around the

301   fixed mean). Note that the quantity of $N_{gainmix}$ reflects the total of N gain in mixture compared

302 to grass monoculture, but it does not allow quantification of the individual contributions from

303 symbiotic N_2 fixation, N transfer, more efficient exploitation of soil and fertilizer N, or N

304 from any other source (e.g. decaying roots of legumes).

305

306 *iv) Relating N data to productivity and climate*

307 To evaluate patterns in N dynamics over the environmental gradient, realized legume

308 proportion, N_{tot} in mixture, and the relative N yield gain in mixture $(N_{gainmix/Ntot}$ in mixture)

309 were related to site productivity, annual precipitation, mean annual temperature, and

310 minimum and maximum temperature. To increase the robustness of results, these calculations

311 were based on site means across the first two experimental years. For consistent comparison,

312 both N_{tot} in mixture and the relative N gain were calculated for a mixture with one third

313 proportion of legumes and two thirds of grasses using a simplified linear mixed model (eqn.

314 S2, Appendix A, Supporting Information). The ratio of proportions (1/3:2/3 legumes:grasses)

315 could be justified from analyses that regressed N_{tot} on legume proportion (see results), and

316 amounts of total N yield for this representative mixture are hereafter termed $N_{totmix.}$ Site

317 productivity was estimated by averaging across the biomass yields of all grass monocultures.

318 Pearson correlation and ordinary least squares regression were used to quantify relationships

319 between legume proportion, $N_{totmix,}$ the relative N yield gain in mixture $(N_{gainmix/Ntotmix)},$ and

320 environmental variables (predictors) (see Table S4, Supporting Information, for site values of

321 the five environmental variables, and Table S5 for their correlation matrix). All analyses were

322 performed with the statistics software R (R Development Core Team, 2014).

323 **Results**

324 *Positive effect of grass-legume mixtures on total N yield*

325 Annual N_{tot} of mixtures was significantly greater than that of grass monocultures at the

326 majority of evaluated sites in all years (Fig. 3), and approximated the values of N_{tot} in legume

327 monocultures. In general, the positive mixture effect on N_{tot} was already evident in the first

328 year and persisted over years (Fig. 3); however, no mixture effect on N_{tot} was apparent at the

329 continental sites Lithuania_a (site 18; years 1 and 2), and Poland_a and Poland_b (sites 26

330 and 27, all years).

331

332 *Positive effect of legume proportion on total N yield and N yield gain in mixture*

333 N_{tot} was significantly affected by the proportion of legumes in mixtures. The effects of

334 legume proportion on N_{tot} were similar across years and were maximal when legume

335 proportions in mixtures were 60, 42, and 40% in years 1, 2, and 3, respectively (Fig. 4). More

336 importantly, because N_{tot} was non-linearly affected by legume proportion (Table 2, Table S6,

337 Supporting Information), N_{tot} was not significantly different from the maximum amount in

338 mixture over a wide range of legume proportion (Fig. 4), and mixtures with approximately

339 one third proportion of legumes attained 95% of maximum N_{tot} (Table 3). N_{tot} varied

340 substantially among sites, with greatest (predicted) values in mixtures being as high as 480 kg

341 N ha^{-1} year^{-1} at Ireland (site 15, year 1) and Switzerland (site 34, year 2), and smallest values

342 in Iceland (sites 13 and 14: around 50 kg N ha^{-1} year^{-1}; Fig. 4).

343

344 Because mixtures with approximately one third proportion of legumes attained 95% of the

345 maximum N_{tot} (Table 3), further values for N_{tot} $^{(and}$ $_{Ngainmix,}$ below) were estimated for this

346 representative mixture (1/3:2/3 legumes:grasses). Doing so, N_{totmix} decreased by 12% between

16

347 years 1 and 2 ($P = 0.09$ for the difference in $_{Ntotmix)}$, and by a further 23% between years 2

348 and 3 ($P = 0.002$).

349

350 Average annual values of $_{Ngainmix}$ across sites ranged between 108 and 76 kg ha$^{~1}$ year$^{~1}$ (years

351 1 and 3, respectively), resulting in a relative N gain in mixture $_{(Ngainmix/Ntotmix)}$ that exceeded

352 0.3 in all years (Table 3). Again, there was a large variation of $_{Ngainmix}$ among sites with

353 amounts being as high as 280 kg ha$^{~1}$ year$^{~1}$ at Norway_a (site 22, year 1) but being close to

354 zero at the continental sites Lithuania _a (site 18, year 1), Poland_a, and Poland_b (sites 26

355 and 27, all years), suggesting that at these sites legumes in mixtures induced no gain in total N

356 yield (Fig. 4). Compared to grass pure stands and averaged across sites, $_{Ngainmix}$ was 61, 46,

357 and 64% in years 1, 2, and 3, respectively (calculated from Table 3), meaning that mixtures

358 with only one third proportion of legumes had, on average across all years, 57% higher total

359 N yield than pure grass stands.

360

361 *Declining stand legume proportion over time*

362 Realized legume proportion in stands decreased generally over time: across sites, realized

363 legume proportion was 32, 21, and 9% in years 1, 2, and 3, respectively. Regarding site

364 variation, almost the full possible range was covered: over all swards with highly varying

365 legume proportion in the seed mixture, Norway_a (site 22), Wales_a (35), and Switzerland

366 (34) still had average legume proportions around 50% and maximal values exceeding 80% by

367 year 2; in contrast, legumes were absent from the second year onward in Ireland (15) and had

368 disappeared by the third year in Lithuania_c (20) (Fig. 4).

369

370 *Strong effect of minimum site temperature on legume proportion and N yield gain in mixture*

371　　Out of the environmental variables tested, minimum site temperature most strongly impaired

372 both stand legume proportion and the relative N yield gain in mixture (Fig. 5a & c), indicating

373 that harsh environmental conditions hampered legumes' growth (see Table S4, Supporting

374 Information, for site values of $_{Ntotmix}$ ^{and} $_{Ngainmix,}$ and Table S7 for a summary of all

375 regressions). Although to a weaker degree, low annual precipitation also impacted on both

376　　　　stand legume proportion and $_{Ngainmix/Ntotmix}$ (Fig. 5b & d). The parallel responses of legumes'

377 growth and relative N yield gain in mixture to these environmental parameters suggested that

378 legume proportion and $_{Ngainmix/Ntotmix}$ were correlated to each other, which indeed was the

379　　case (Fig. 5e). Furthermore, $_{Ntotmix}$ was positively correlated to site productivity ($R = 0.703$, P

380 = 0.003, Table S7, Supporting Information); however, the relative N yield gain in mixture was

381　　not (Fig. 5f). Taken together, this suggested that the relative N gain of a balanced grass-

382 legume mixture was affected more by climatic conditions than by the largely differing

383　　productivity levels across sites.

384 **Discussion**

385 Mixing grasses and legumes in agricultural grassland systems yielded considerable benefits to

386 total N yield $_{(Ntot)}$ over a wide range of European environmental conditions. Averaged across

387 sites and years, mixtures with only one third proportion of legumes had as much as 57%

388   higher $_{Ntot}$ than pure grass stands, indicating a substantial N yield gain in mixtures ($N_{gainmix}$),

389 and such mixtures attained amounts of $_{Ntot}$ close to the maximum $_{Ntot}$ acquired by any stand.

390   The relative N yield gain in mixture $_{(Ngainmix/Ntotmix)}$ was not affected by the great differences

391   in productivity levels across sites. This clearly highlights the potential of grass-legume

392 mixtures as a practical management option for sustainable agriculture, and we elaborate on a

393 number of relevant points below.

394

395 *Substantial N yield advantage in mixtures as compared to grass monocultures*

396 Amounts of $_{Ntot}$ from the temperate sites of this study corresponded well to reports from

397 comparable grass-legume systems (Boller & Nösberger, 1987; Høgh-Jensen & Schjoerring,

398   1997; Carlsson & Huss-Danell, 2003 for review); however, this experiment also covered

399   pedo-climatic regions that have been poorly investigated so far. At the majority of sites, the

400 positive mixture effect on $_{Ntot}$ appeared in the first year, was maintained for the second year

401   and was still apparent in five out of eight evaluated sites in the third year. In particular, the

402 positive mixture effect on $_{Ntot}$ was apparent also at the Nordic sites in Iceland (sites 13 and

403   14) and Tromsø-Norway (site 23), where absolute amounts of $_{Ntot}$ (Fig. 3) and harvested

404 biomass yield (Finn *et al.*, 2013) were comparably small. Highest N advantages of mixtures

405 *versus* grass monocultures were more than 250 kg N ha^{-1} year^{-1} (Belgium (site 1), Switzerland

406 (site 34), Fig. 3a) and resulted in a more than two-fold higher N output in mixed swards than

407   in stands with grass only. Considering the substantial differences in site productivity, ranging

408 between 2 and 14 t DM ha^{-1} year^{-1}, and the substantial gain of $_{Ntot}$ in mixtures across sites and

409 years, the data clearly demonstrate the great potential of mixed grass-legume swards for

410 resource-efficient production in varying pedo-climatic conditions. Using mixed swards

411   instead of pure grass stands, more N yield can be expected for a given amount of N fertilizer

412   applied. Alternatively, if the aim is to reduce fertilizer N application for financial, regulatory

413   and/or environmental reasons (Godfray *et al.*, 2010), our data show the potential to do so

414 without necessarily compromising N yield and total harvested biomass (Nyfeler *et al.*, 2009;

415   Finn *et al.*, 2013).

416

417 *N yield gain in mixtures is evident over a wide range of legume proportion*

418 With few exceptions, $_{Ntot}$ demonstrated a considerable increase with increasing legume

419 proportion up to about 30%. With higher percentages of legumes, $_{Ntot}$ did not continue to

420   increase (Fig. 4). This is a highly valuable result. It indicates that almost all (~95%) of the

421 maximum benefit to $_{Ntot}$ from mixing grasses and legumes can be achieved with a modest

422 (~30%) legume proportion in the mixture. Moreover and equally relevant, the wide range for

423 which $_{Ntot}$ was not different from maximum values indicates that the benefits of legumes

424 regarding N gain in mixtures can prevail despite considerable fluctuations of grass-legume

425 proportions in swards. This is an important feature for practical grassland management.

426

427 The method to compute $_{Ngainmix}$ has been previously used to calculate the amount of apparent

428   symbiotic N_2 fixation $_{(Nsym)}$ in grass-legume mixtures ("N-difference method", e.g. Ledgard

429 & Steele, 1992). The N-difference method has been criticized because it assumes that the

430 cumulative uptake of N from soil and fertilizer of grasses and legumes in mixture would be

431   the same as for the reference grass monoculture, which may not necessarily be the case

432 (Boller & Nösberger, 1987; Nyfeler *et al.*, 2011). The N-difference method therefore can lead

433   to biased estimates of $_{Nsym.}$ At the Swiss site of our study, Nyfeler *et al.* (2011) used the more

434 accurate ^{15}N dilution procedure to quantify N_{tot}, N_{sym} and N uptake from non-symbiotic

435   sources (N derived from the soil and/or fertilizer). They demonstrated that, for a 50:50

436 mixture of grasses and legumes and fertilizer N application of 50 kg ha^{-1} year^{-1}, the N-

437 difference calculation resulted in N_{sym} values that were 26% higher than the ^{15}N dilution

438 procedure in the first production year, while overestimation was 24% in the second year. For

439 N applications of 150 kg ha^{-1} year^{-1}, overestimation by the N-difference method became

440 smaller, and was 17% and 7% in the first and second year, respectively. In Northern mixed

441 grassland (Vågønes, Norway, 69°N) of *Trifolium repens* L. (white clover) and *Phleum*

442 *pratense* L. (timothy) and no fertilizer N application, the overestimation of the N-difference

443 method compared to ^{15}N dilution was 7% and 36% in two production years, respectively

444 (Nesheim & Oyen, 1994). Regarding our continental-scale experiment, $N_{gainmix}$ corresponds to

445   values of the N-difference calculation. It is important to realize that this difference method is

446 appropriate to the purpose used here, namely to measure the total N gain achieved by the

447 grass-legume mixture compared to the pure grass stand. However, because it appears that this

448 difference method does not allow quantification of symbiotic N_2 fixation and N uptake from

449 non-symbiotic sources with acceptable accuracy, we do not aim to specify these different

450   fractions. Nevertheless, the above assessment suggests that the great majority of $N_{gainmix}$ is

451   derived from N_2 fixation of legumes (see also Boller & Nösberger, 1987; Zanetti *et al.*, 1997).

452

453 This gain of N yield in mixtures due to the presence of legumes can be explained by various

454 mechanisms. A linear increase in N_{tot} with increasing legume proportion can be expected

455   solely through a constant additional N input to the system per unit of legumes (see B in Fig.

456 2). However, because we found a highly nonlinear response of N_{tot} (Fig. 4, C in Fig. 2),

457 positive interactions between grasses and legumes must have played a role, and we suggest

458   four possible mechanisms. First, positive mixing effects have been attributed to stimulation of

21

459 the rate of symbiotic N_2 fixation by N-demand from co-occurring grasses (Høgh-Jensen &

460   Schjoerring, 1997). At the Swiss site, Nyfeler *et al.*(201 1) revealed that the rate (%) of N

461   derived from symbiosis in the clover plants was significantly higher at low legume proportion

462 than at high legume proportion. This pattern could be explained by strong competition from

463 grasses for N from non-symbiotic sources: even if the grass component in mixture was

464 reduced to 50%, grass still took up the same total amount of N from non-symbiotic sources as

465 a stand with 100% grass (Nyfeler *et al.*, 2011). Second, the presence of both grass and legume

466 components in mixture allows for transfer of symbiotically fixed N from legumes to grasses.

467 Quantification of apparent N transfer between legumes and grasses shows that more than 40%

468 of the N nutrition of the grass component in mixture can derive from N_2 fixation of the

469   legumes (Boller & Nösberger, 1987; Høgh-Jensen & Schjoerring, 1997; Nyfeler *et al.*, 2011)

470 with N transfer being greatest in equilibrated mixtures. Third, the nonlinear response of N_{tot}

471   can be attributed to an increased capability of the grass component to acquire N from non-

472   symbiotic sources in the presence of legumes (Nyfeler *et al.*, 2011). Grasses have a denser

473   root system (i.e. comparably higher root length density per unit soil volume and high root

474   surface area of active absorption, Craine *et al.*, 2002; Hill *et al.*, 2006), which they use to

475   outcompete the legumes in accessing the available mineral and fertilizer N. Fourth, increased

476   utilization of fertilizer and soil N resources through temporal and/or spatial niche

477 complementarity between grasses and legumes can also explain the positive mixing effects

478   (van Ruijven & Berendse, 2005; Mueller *et al.*, 2013). To conclude, the benefit of mixed

479 swards to total N output of the system comes through significant grass-legume interactions.

480

481   Considerable mixing effects on total harvested biomass yield have recently been shown for

482   the same sites as presented here (Finn *et al.*, 2013). Finn *et al.* (2013) found transgressive

483   overyielding (mixtures outperformed highest yielding monocultures, Trenbath, 1974) of about

484 20% (excluding weed biomass), indicating that mixtures produced more biomass than either

485 grass *or* legume monocultures. To maximise both total N output and total biomass yield,

486 while at the same time minimising the risk of N losses to the environment (Loiseau *et al.*,

487 2001; Nyfeler, 2009), we infer that an optimal range of legume proportion in mixtures should

488 range between 30-50%.

489

490 *Legume proportion and N yield gain in mixtures are affected by environmental conditions*

491 We found evidence that legume proportion and the relative N yield gain in mixture were

492 negatively correlated with minimum winter temperatures and low annual precipitation (Fig.

493   5). Much of the evidence on restrictions of legumes' growth and symbiotic N_2 fixation comes

494 from studies in growth chambers (e.g. Nesheim & Boller, 1991; Lynch & Smith, 1993; Zhang

495 *et al.*, 1995; Serraj & Sinclair, 1996). Low temperature seems to hamper N_2 fixation more

496 than plant growth (Lynch & Smith, 1993; Zhang *et al.*, 1995; Hartwig, 1998), and severe

497   drought has been shown to disrupt nodule activity (Serraj & Sinclair, 1996; Serraj *et al.*,

498   1999). Such results accord with our findings gained under field conditions. However, because

499 we did not directly measure symbiotic activity of legumes but demonstrated impacts of

500 climate on legume proportion, we cannot conclude whether harsh climatic conditions

501 impacted more on symbiotic N_2 fixation or on legume plant growth.

502

503 A decrease in the legume proportion of mixed swards can be induced by application of N

504   fertilizers. For example, in a related experiment (Nyfeler *et al.*, 2009), different levels of

505 mineral N were applied to grass-clover mixtures (two grass and two clover species) managed

506   for three consecutive years. Here, N fertilization significantly affected clover proportion:

507   averaged across years and all mixtures receiving 50 kg N ha^{-1} year^{-1}, the two clover species

508 (sum of both) achieved 41% proportional biomass; however, fertilized with 150 kg N ha^{-1}

509   year^{-1}, clover proportions were only 31% (Nyfeler *et al.*, 2009). Comparable results were

510 found by Nassiri & Elgersma (2002), where in only one growing season the application of

511   150 kg N ha^{-1} year^{-1} reduced the clover content in dry matter harvest of perennial ryegrass-

512 white clover mixtures to 12% compared to 43% without N application. Such impacts of N

513   fertilizers on legume persistence might be of less importance in temporary grassland as part of

514 crop rotation systems, where swards are generally maintained for a seeding year and one or

515 two production years before they are ploughed for growing cereal crops. Moreover, although

516 legumes might decrease over time, their positive effect on mixture biomass yield can still be

517   prevalent even at low proportion (Nyfeler *et al.*, 2009; Finn *et al.*, 2013), which can be

518   explained by strong grass-legume interactions as outlined above or by legacy effects (e.g.

519 release of fixed N from decaying roots or increased N pools in soil organic matter derived

520 from N$_2$ fixation of preceding years).

521

522 In our experiment, positive effects of legumes on $_{Ntot}$ were still evident in year two and three

523   (Fig. 3) despite the successive decrease in legume proportion, and mixtures with only one

524 third proportion of legumes provided a significant gain in N yield as compared to pure grass

525 stands (Fig. 4). This proves our experiment with sown temporary grassland to be a good

526 model system to study the relation between legume proportions realized in the sward and N

527 yield gain in mixtures, and this relation holds also for permanent grassland as long as

528 adequate proportions of legumes can be maintained. Yet, the persistence of legumes in

529 permanent grassland is challenging (Guckert & Hay, 2001), and our study implies that

530 research should focus on sward management strategies to stabilize legume proportion under

531   varying climatic conditions. Evidence suggests that adjusting N fertilizer rates and defoliation

532 frequency can increase the abundance of white clover (Schwank *et al.*, 1986; Hebeisen *et al.*,

533   1997; Lüscher *et al.*, 2014), the most important legume species in permanent grassland of

24

534 temperate regions (Guckert & Hay, 2001). In our experiment, no specific actions were

535 undertaken to counteract legume decrease. Also, realized legume proportion was not

536   correlated to N application rates at sites ($R = –0.219$, $P = 0.434$), indicating that

537 environmental conditions were more decisive for legume performance than N fertilizers at

538   rates applied in our experiment.

539

540   Four sites merit further consideration. At the three continental sites Lithuania_a, Poland_a,

541   and Poland_b, legumes established well in the first year and reached proportions of up to

542 96%; nevertheless, N gains in mixture were not evident (Fig. 4a) suggesting that symbiotic N_2

543   fixation of the clovers did not work properly. We suspect that in concert with low winter

544 temperatures hampering nodulation (Hartwig, 1998), lack of *Rhizobia* species in soils of the

545 agronomically improved grassland may have prevented legumes to form an efficient

546   symbiotic relationship. The situation is different for Ireland, where legumes were present and

547 mixtures realized high gains of total N yield in the first year, but legumes disappeared from

548 the second year onward. With minimum temperatures of -1° C, annual precipitation around

549 the mean of the investigated range (932 mm year$^{~1}$), and comparably high site productivity

550   (14.1 t DM ha$^{~1}$ year$^{~1}$), climatic conditions cannot be responsible for the strong legume

551   decline. Because the Irish site had been a highly fertilized monoculture of *L. perenne*

552 grassland for many years preceding the experiment, we speculate that the very high soil

553   fertility and resulting high competition from the grasses during the experiment may have

554 hampered the legumes' growth (Schwank *et al.*, 1986). Also, Ireland had a soil pH of 5.3,

555 being the lowest among sites (Kirwan *et al.*, 2014), which may have hampered the growth of

556 the two legume species.

557

558   Taken together, it can be concluded that climatic conditions and the natural presence of

559 *Rhizobia* species define a boundary for the performance of legumes, in particular for the

560   species used in this experiment. Our results indicate the need for specific research on how

561 legumes can be maintained in mixed swards under varying and extreme climatic conditions.

562 Besides the inoculation of soil with *Rhizobia* species and the optimization of sward

563 management through adjusted N fertilizer inputs and defoliation frequencies, research should

564 focus on breeding of adapted cultivars and selection of different legume species that would

565 withstand low winter temperatures and/or severe drought.

566

567   *Wider implications: Legumes as a key contributor to sustainable intensification of grassland*

568 *across largely differing productivity levels*

569 One important result of this study is that, although $_{Ntot}$ in mixture was strongly affected by

570   site productivity, the relative N yield gain in mixture was not (Fig. 5f). This means that less

571   productive sites as well as more productive sites can equally profit from grass-legume

572 mixtures to increase N output. Adaptation of legumes to differing productivity levels can be

573 explained by the concept of "N_2 fixation regulation by demand" (Hartwig, 1998; Soussana &

574 Tallec, 2010). Following Hartwig (1998), the degree of symbiotic N_2 fixation of legumes is

575 controlled by a series of eco-physiological triggers and N feedback mechanisms from the

576 individual plant to the ecosystem level, with N_2 fixation of legumes being largely regulated by

577 the N sink strength (N-demand) of the whole system (Hartwig, 1998; Soussana & Tallec,

578 2010; Lüscher *et al.*, 2011). It has been shown repeatedly that, under low to medium N

579   fertilizer supply (< 100 kg N ha^{-1} year^{-1}), legumes in a balanced mixture with grasses acquire

580 the large majority of their N nutrition through symbiotic N_2 fixation (Nesheim & Oyen, 1994;

581   Høgh-Jensen & Schjoerring, 1997; Nyfeler *et al.*, 2011). In contrast, as amounts of N

582 fertilizer increase, a decline of N acquired from symbiosis in legumes has been demonstrated

583   in fi

584 studies and mathematical models (Soussana *et al.*, 2002; Soussana & Tallec, 2010). Such

585 propensity to buffer N supply can also be an advantage for protecting water quality as long as

586 N fertilizer application is not too high, and provided that the grass component in mixture is

587   sufficiently large (> 30%) so that nitrate absorption can occur before the water leaches out of

588   the root zone (Eriksen *et al.*, 2004). Indeed, with high application of N fertilizers (> 400 kg N

589 ha^{-1} $year^{-1}$) or high legume proportion in swards (> 70%), considerable N leaching can been

590   observed (Loiseau *et al.*, 2001; Ledgard *et al.*, 2009; Nyfeler, 2009). Thus, there are at least

591 three reasons why N losses from legume-based grassland systems should be lower than from

592   fertilized grass systems: (i) nitrogen is fixed symbiotically within the legume nodules and thus

593   is not freely available in the soil in a reactive form, (ii) symbiotic N_2 fixation activity is down-

594 regulated if the sink of N for plant growth is small, and (iii) in balanced grass-legume

595 mixtures, the grass roots take up N derived from legumes and from mineralization of soil

596 organic matter.

597

598   Sustainable intensification and food security are required for a wide range of agricultural

599   systems (Godfray *et al.*, 2010) but the focus of action might differ among intensity levels of

600   agricultural production. In less productive systems, such as those at the arctic sites in our

601   study, savings in N fertilizer are probably of less importance due to low levels of N fertilizer

602 use; however, our data show that for a given amount of N fertilizer input, higher N output

603      $(N_{tot}$ or forage protein per unit area) can be expected with grass-legume mixtures than with

604 pure grass alone (more output for the same input). In systems that use high levels of N

605   fertilizer to achieve high production levels, in contrast, the same N output can be achieved by

606 mixed swards with less input of N fertilizer (the same output from less input), thereby

607   reducing energy use (Kitani *et al.*, 1999), nitrate losses (Jensen *et al.*, 2012), and GHG

608   emissions (Davidson, 2009; Schils *et al.*, 2013; Schmeer *et al.*, 2014).

609

610 Finally, the use of grass-legume mixtures fulfills recent demands for climate change

6ll   mitigation (Smith *et al.*, 2013). The savings in application of N fertilizers that can potentially

612 be achieved through the benefit of symbiotic N_2 fixation in grassland mixtures makes their

613 use an important strategy for reduction of GHG emissions from agriculture (Smith *et al.*,

614   2008; Canfield *et al.*, 2010). Therefore, the use of such mixtures should be actively supported

615 as they also meet major targets of practical grassland management, such as robustness in

616   gains of total biomass yield despite variation in legume proportions, and applicability across

617   wide environmental gradients (Suter *et al.*, 2012; Finn *et al.*, 2013). Provided that cultural

618 barriers can be overcome (e.g. lacking knowledge in establishment of mixtures and their

619 management to improve legume persistence), grass-legume mixtures are also a mitigation

620 measure that can be implemented in the near future (Smith & Gregory, 2013; Smith *et al.*,

621   2013). This is an important feature to meet the urgent needs for reductions in GHG emissions

622 from the agriculture, forestry and other land use sector, which are estimated to be around 25%

623   of total anthropogenic GHG output (Bustamante *et al.*, 2014).

624

625 In conclusion, this study demonstrates that the N output of forage harvest is maximized in

626 mixtures over a wide range of production levels. In the face of high economic and

627   environmental costs of industrial N fertilizers (Kitani *et al.*, 1999; Gruber & Galloway, 2008;

628   Canfield *et al.*, 2010), the contribution of symbiotic N_2 fixation by legumes to grassland N

629 supply appears to be a key strategy to maintain and increase current levels of production and

630 protein self-sufficiency in a more sustainable way than achieved so far.

References

Barton L, Butterbach-Bahl K, Kiese R, Murphy DV (2011) Nitrous oxide fluxes from a grain-legume crop (narrow-leafed lupin) grown in a semiarid climate. *Global Change Biology*, **17**, 1153-1166.

Boller BC, Nösberger J (1987) Symbiotically fixed nitrogen from field-grown white and red clover mixed with ryegrasses at low levels of ^{15}N-fertilization. *Plant and Soil*, **104**, 219-226.

Bustamante M, Robledo-Abad C, Harper R *et al.* (2014) Co-benefits, trade-offs, barriers and policies for greenhouse gas mitigation in the agriculture, forestry and other land use (AFOLU) sector. *Global Change Biology*, **20**, 3270-3290.

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of earth's nitrogen cycle. *Science*, **330**, 192-196.

Carlsson G, Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil*, **253**, 353-372.

Cassman KG, Dobermann A, Walters DT (2002) Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio*, **31**, 132-140.

Cornell JA (2002) *Experiments with mixtures*. Wiley, New York, US.

Craine JM, Wedin DA, Chapin FS, Reich PB (2002) Relationship between the structure of root systems and resource use for 11 North American grassland plants. *Plant Ecology*, **165**, 85-100.

Davidson EA (2009) The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature Geoscience*, **2**, 659-662.

Ecoinvent Centre (2010) The life cycle inventory data, v2.2. Swiss Centre for Life Cycle Inventories, Dübendorf. ISBN 3-905594-38-2. www.ecoinvent.org (accessed 20 January 2015).

Eriksen J, Vinther FP, Soegaard K (2004) Nitrate leaching and N$_2$-fixation in grasslands of different composition, age and management. *Journal of Agricultural Science*, **142**, 141-151.

Finn JA, Kirwan L, Connolly J *et al.* (2013) Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. *Journal of Applied Ecology*, **50**, 365-375.

Foley JA, DeFries R, Asner GP *et al.* (2005) Global consequences of land use. *Science*, **309**, 570-574.

672 Foley JA, Ramankutty N, Brauman KA *et al.* (2011) Solutions for a cultivated planet. *Nature*,
673   **478**, 337-342.

674 Frey E (1955) Neue Standardmischungen für den Futterbau. *Mitteilungen für die*
675   *Schweizerische Landwirtschaft*, **3**, 129-142.

676 Galloway JN, Townsend AR, Erisman JW *et al.* (2008) Transformation of the nitrogen cycle:
677   Recent trends, questions, and potential solutions. *Science*, **320**, 889-892.

678 Godfray HCJ, Beddington JR, Crute IR *et al.* (2010) Food security: the challenge of feeding 9
679   billion people. *Science*, **327**, 812-818.

680 Gruber N, Galloway JN (2008) An Earth-system perspective of the global nitrogen cycle.
681   *Nature*, **451**, 293-296.

682 Guckert A, Hay RKM (2001) The overwintering, spring growth, and yield in mixed species
683   swards, of white clover in Europe - Preface. *Annals of Botany*, **88**, 667-668.

684 Hartwig UA (1998) The regulation of symbiotic N_2 fixation: A conceptual model of N
685   feedback from the ecosystem to the gene expression level. *Perspectives in Plant Ecology*
686   *Evolution and Systematics*, **1**, 92-120.

687 Hebeisen T, Lüscher A, Zanetti S *et al.* (1997) Growth response of *Trifolium repens* L. and
688   *Lolium perenne* L. as monocultures and bi-species mixture to free air CO_2 enrichment
689   and management. *Global Change Biology*, **3**, 149-160.

690 Hill JO, Simpson RJ, Moore AD, Chapman DF (2006) Morphology and response of roots of
691   pasture species to phosphorus and nitrogen nutrition. *Plant and Soil*, **286**, 7-19.

692 Høgh-Jensen H, Schjoerring JK (1994) Measurement of biological dinitrogen fixation in
693   grassland - comparison of the enriched ^{15}N dilution and the natural ^{15}N abundance
694   methods at different nitrogen application rates and defoliation frequencies. *Plant and*
695   *Soil*, **166**, 153-163.

696 Høgh-Jensen H, Schjoerring JK (1997) Interactions between white clover and ryegrass under
697   contrasting nitrogen availability: N_2 fixation, N fertilizer recovery, N transfer and water
698   use efficiency. *Plant and Soil*, **197**, 187-199.

699 IPCC (2006) *Guidelines for national greenhouse gas inventories*. Intergovernmental Panel of
700   Climate Change, Tokyo, Japan. www.ipcc-nggip.iges.or.jp/public/index.html (accessed
701   20 January 2015).

702 Jensen ES, Peoples MB, Boddey RM, Gresshoff PM, Hauggaard-Nielsen H, Alves BJR,
703   Morrison MJ (2012) Legumes for mitigation of climate change and the provision of
704   feedstock for biofuels and biorefineries. A review. *Agronomy for Sustainable*
705   *Development*, **32**, 329-364.

706 Johnson PO, Neyman J (1936) Tests of certain linear hypotheses and their application to some

707   educational problems. *Statistical Research Memoirs*, **1**, 57-93.

708 Kenward MG, Roger JH (1997) Small sample inference for fixed effects from restricted

709   maximum likelihood. *Biometrics*, **53**, 983-997.

710 Kirwan L, Lüscher A, Sebastia MT *et al.* (2007) Evenness drives consistent diversity effects

711   in intensive grassland systems across 28 European sites. *Journal of Ecology*, **95**, 530-539.

712 Kirwan L, Connolly J, Brophy C *et al.* (2014) The Agrodiversity Experiment: three years of

713   data from a multisite study in intensively managed grasslands. *Ecology*, **95**, 2680.

714 Kitani O, Jungbluth T, Peath RM, Ramdani A (eds) (1999) *CIGR handbook of agricultural*

715   *engineering. Volume V: Energy & Biomass Engineering*, American Society of

716   Agricultural Engineers, St Joseph, US.

717 Ledgard S, Schils R, Eriksen J, Luo J (2009) Environmental impacts of grazed clover/grass

718   pastures. *Irish Journal of Agricultural and Food Research*, **48**, 209-226.

719 Ledgard SF, Steele KW (1992) Biological nitrogen fixation in mixed legume-grass pastures.

720   *Plant and Soil*, **141**, 137-153.

721 Lee ES, Forthofer RN (2006) *Analyzing Complex Survey Data*. SAGE Publications, Thousand

722   Oaks, US.

723 Loiseau P, Carrere P, Lafarge M, Delpy R, Dublanchet J (2001) Effect of soil-N and urine-N

724   on nitrate leaching under pure grass, pure clover and mixed grass/clover swards.

725   *European Journal of Agronomy*, **14**, 113-121.

726 Lüscher A, Soussana JF, Huguenin-Elie O (2011) Role and impacts of legumes in grasslands

727   for high productivity and N gain from symbiotic N_2 fixation. In: *Grassland Productivity*

728   *and Ecosystem Services* (eds Lemaire G, Hodgson J, Chabbi A), pp. 101-107. CABI,

729   Oxfordshire, UK.

730 Lüscher A, Mueller-Harvey I, Soussana JF, Rees RM, Peyraud JL (2014) Potential of legume-

731   based grassland-livestock systems in Europe: a review. *Grass and Forage Science*, **69**,

732   206-228.

733 Lynch DH, Smith DL (1993) Soybean (*Glycine max*) nodulation and N_2-fixation as affected

734   by exposure to a low root-zone temperature. *Physiologia Plantarum*, **88**, 212-220.

735 Mueller KE, Tilman D, Fornara DA, Hobbie SE (2013) Root depth distribution and the

736   diversity-productivity relationship in a long-term grassland experiment. *Ecology*, **94**, 787-

737   793.

738 Nassiri M, Elgersma A (2002) Effects of nitrogen on leaves, dry matter allocation and

739   regrowth dynamics in *Trifolium repens* L. and *Lolium perenne* L. in pure and mixed

740   swards. *Plant and Soil*, **246**, 107-121.

741 Nesheim L, Boller BC (1991) Nitrogen-fixation by white clover when competing with grasses

742   at moderately low temperatures. *Plant and Soil*, **133**, 47-56.

743 Nesheim L, Oyen J (1994) Nitrogen fixation by red clover (*Trifolium pratense* L.) grown in

744   mixtures with timothy (*Phleum pratense* L.) at different levels of nitrogen fertilization.

745   *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, **44**, 28-34.

746 Nyfeler D (2009) Productivity and nitrogen utilization in productive grassland: effect of

747   species combinations, species proportions and nitrogen fertilization. PhD thesis. ETH

748   Zürich, Zürich.

749 Nyfeler D, Huguenin-Elie O, Suter M, Frossard E, Connolly J, Lüscher A (2009) Strong

750   mixture effects among four species in fertilized agricultural grassland led to persistent

751   and consistent transgressive overyielding. *Journal of Applied Ecology*, **46**, 683-691.

752 Nyfeler D, Huguenin-Elie O, Suter M, Frossard E, Lüscher A (2011) Grass-legume mixtures

753   can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen

754   uptake from symbiotic and non-symbiotic sources. *Agriculture Ecosystems &*

755   *Environment*, **140**, 155-163.

756 Peyraud JL, Le Gall A, Lüscher A (2009) Potential food production from forage legume-

757   based-systems in Europe: an overview. *Irish Journal of Agricultural and Food Research*,

758   **48**, 115-135.

759 Pinheiro JC, Bates DM (2009) *Mixed-effects Models in S and S-Plus*. Springer, New York,

760   US.

761 Pirhofer-Walzl K, Rasmussen J, Høgh-Jensen H, Eriksen J, Søegaard K, Rasmussen J (2012)

762   Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species

763   grassland. *Plant and Soil*, **350**, 71-84.

764 R Development Core Team (2014) *R: A language and environment for statistical computing*.

765   R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0,

766   http://www.R-project.org.

767 Rasmussen J, Gylfadottir T, Loges R, Eriksen J, Helgadottir A (2013) Spatial and temporal

768   variation in N transfer in grass-white clover mixtures at three Northern European field

769   sites. *Soil Biology & Biochemistry*, **57**, 654-662.

770 Rochette P, Janzen HH (2005) Towards a revised coefficient for estimating N_2O emissions

771   from legumes. *Nutrient Cycling in Agroecosystems*, **73**, 171-179.

772 Schils RLM, Eriksen J, Ledgard SF *et al.* (2013) Strategies to mitigate nitrous oxide

773   emissions from herbivore production systems. *Animal*, **7**, 29-40.

774 Schmeer M, Loges R, Dittert K, Senbayram M, Horn R, Taube F (2014) Legume-based

775   forage production systems reduce nitrous oxide emissions. *Soil & Tillage Research*, **143**,

776   17-25.

777 Schwank O, Blum H, Nösberger J (1986) The influence of irradiance distribution on the

778   growth of white clover (*Trifolium repens* L.) in differently managed canopies of

779   permanent grassland. *Annals of Botany*, **57**, 273-281.

780 Serraj R, Sinclair TR (1996) Inhibition of nitrogenase activity and nodule oxygen

781   permeability by water deficit. *Journal of Experimental Botany*, **47**, 1067-1073.

782 Serraj R, Sinclair TR, Purcell LC (1999) Symbiotic N_2 fixation response to drought. *Journal*

783   *of Experimental Botany*, **50**, 143-155.

784 Smith P, Martino D, Cai Z *et al.* (2008) Greenhouse gas mitigation in agriculture.

785   *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 789-813.

786 Smith P, Gregory PJ (2013) Climate change and sustainable food production. *Proceedings of*

787   *the Nutrition Society*, **72**, 21-28.

788 Smith P, Haberl H, Popp A *et al.* (2013) How much land-based greenhouse gas mitigation can

789   be achieved without compromising food security and environmental goals? *Global*

790   *Change Biology*, **19**, 2285-2302.

791 Soussana JF, Minchin FR, Macduff JH, Raistrick N, Abberton MT, Michaelson YT (2002) A

792   simple model of feedback regulation for nitrate uptake and N_2 fixation in contrasting

793   phenotypes of white clover. *Annals of Botany*, **90**, 139-147.

794 Soussana JF, Tallec T (2010) Can we understand and predict the regulation of biological N_2

795   fixation in grassland ecosystems? *Nutrient Cycling in Agroecosystems*, **88**, 197-213.

796 Suter D, Rosenberg E, Mosimann E, Frick R (2012) Standardmischungen für den Futterbau -

797   Revision 2013-2016. *Agrarforschung Schweiz*, **3**, 1-12.

798 Suter M, Ramseier D, Güsewell S, Connolly J (2007) Convergence patterns and multiple

799   species interactions in a designed plant mixture of five species. *Oecologia*, **151**, 499-511.

800 Taube F, Gierus M, Hermann A, Loges R, Schönbach P (2014) Grassland and globalization -

801   challenges for northwest European grass and forage research. *Grass and Forage Science*,

802   **69**, 2-16.

803 Temperton VM, Mwangi PN, Scherer-Lorenzen M, Schmid B, Buchmann N (2007) Positive

804   interactions between nitrogen-fixing legumes and four different neighbouring species in a

805   biodiversity experiment. *Oecologia*, **151**, 190-205.

806 Thornton PK (2010) Livestock production: recent trends, future prospects. *Philosophical*

807   *Transactions of the Royal Society B-Biological Sciences*, **365**, 2853-2867.

808 Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability

809   and intensive production practices. *Nature*, **418**, 671-677.

810 Trenbath BR (1974) Biomass productivity of mixtures. In: *Advances in Agronomy* (ed Brady

811   NC), pp. 177-2 10. Academic Press, New York, US.

812   van Ruijven J, Berendse F (2005) Diversity-productivity relationships: Initial effects, long-

813   term patterns, and underlying mechanisms. *Proceedings of the National Academy of*

814   *Sciences of the United States of America*, **102**, 695-700.

815 Zanetti S, Hartwig UA, van Kessel C *et al.* (1997) Does nitrogen nutrition restrict the CO_2

816   response of fertile grassland lacking legumes? *Oecologia*, **112**, 17-25.

817 Zhang F, Lynch DH, Smith DL (1995) Impact of low root temperatures in soybean [*Glycine*

818   *max* (L.) Merr.] on nodulation and nitrogen fixation. *Environmental and Experimental*

819   *Botany*, **35**, 279-285.

820 **Supporting information**

821   Additional Supporting Information may be found in the online version of this article:

822

823 *Site information and details on the experimental design*

824 **Table S1** Site information on the sixteen sites including experimental details

825 **Table S2** Sowing proportions of the two grass and legume species in experimental stands
826

827 *Supporting text and information on the methods and analyses*

828 **Appendix A**

829

830 *Supplementary data*

831   **Table S3** Predicted total nitrogen yield of monocultures and mixing effects of the equi-
832 proportional mixture

833 **Table S4** Site values of environmental variables, total nitrogen yield $_{(Ntot)}$ and N yield gain
834   in mixture ($N_{gainmix}$)

835 **Table S5** Correlation matrix of the five environmental variables recorded in the experiment

836 **Table S6** Regression estimates and their standard errors from the linear mixed model

837 **Table S7** Summary of regressions relating realized legume proportion, total nitrogen yield
838   (N_{tot}), and the relative N yield gain in mixture $_{(Ngainmix/Ntot)}$ to site productivity and four
839   climatic variables

840 **Fig. S1** Fitted lines to total N yield of individual sites as affected by sown legume proportion

841

842 **Tables**

843

844 **Table 1** Identity and functional types of species selected for experimentation in different regions of Europe. The species reflected agronomic

845 importance and the adaptation to pedo-climatic conditions and were selected on the advice of local experts.

			Grass species		Legume species	
Species group		# Sites	Gl[‡]	G2[§]	Ll[‡]	L2[§]
Mid European	(ME)	12	*Lolium perenne* L.	*Dactylis glomerata* L.	*Trifolium pratense* L.	*Trifolium repens* L.
Northern European	(NE)	3	*Phleum pratense* L.	*Poa pratensis* L.	*Trifolium pratense* L.	*Trifolium repens* L.
Other	(O)	1	*Lolium perenne* L.	*Phleum pratense* L.	*Trifolium pratense* L.	*Trifolium repens* L.

846 ‡ _ Fast establishing species: fast germination and fast establishment, thereby providing adequate cover of soil in the sowing year and high biomass

847 yields in the first and second years after sowing. These species often lack persistency.

848 § Temporally persistent species: slower in germination and growth rate during establishment but highly competitive in the long run, therefore

849 increasing in cover and biomass yields over initial years and constituting the majority of yield from the third year onwards.

850

851   **Table 2** Summary of analysis of total N yield $_{(Ntot)}$ regressed on legume proportion

852   (Legume) in the multisite grassland experiment across Europe. *F*-tests refer to the fixed

853   effects of the linear mixed model. See Material and Methods for detailed information on the

854   model.

Variable	Df$_{num}$	Df$_{den}$	*F*-value	*P*
Legume linear (Legume)	1	14.9	19.4	< 0.001
Legume quadratic (Legume^2)	1	15.0	19.4	< 0.001
Legume cubic (Legume^3)	1	14.9	12.0	0.003
Year	2	12.8	9.4	0.003
DeltaBiomass‡	1	749.4	1664.4	< 0.001
Legume x Year	2	10.4	5.6	0.022
Legume x DeltaBiomass	1	730.0	4.7	0.030
DeltaBiomass x Year	2	715.1	7.8	< 0.001

855   ‡ DeltaBiomass is an effect on $_{Ntot}$ that is uncorrelated to the other predictors (see

856 Appendix A, Supporting Information, for details). Seed density was not significant and

857 was omitted. For regression estimates and their standard errors see Table S6, Supporting

858   Information.

859 $_{Dfnum:}$ degrees of freedom of term; $_{Dfden:}$ degrees of freedom of error (which can be

860 fractional in restricted maximum likelihood analysis)

861   **Table 3** Total nitrogen yield $_{(Ntot)}$ in monocultures and grass-legume mixtures and N yield

862   gain $_{(Ngain)}$ in stands containing legumes across all sites of the continental-scale grassland

863   experiment. Values are the fixed estimates (±1 standard error) derived from the linear mixed

864 model corresponding to Table 2 (eqn. S1, Appendix A, Supporting Information, and Table

865   S6). N yield gain in legume pure stands $_{(Ngainleg)}$ and mixtures $_{(Ngainmix)}$ was calculated as the

866 difference between $_{Ntot}$ of a stand and $_{Ntot}$ of the pure grass stand. Values of $_{Ntot}$ in mixture

867   (N$_{totmix}$), N$_{gainmix}$, ^{and} $_{Ngainmix/Ntotmix}$ are predicted for a stand with one third proportion of

868 legumes (L) and two thirds of grasses (G).

a) $_{Ntot}$ (kg ha^{-1} year^{-1})				Minimum legume
	Pure stands		Mixture	proportion to attain
	Grass	Legume‡	1/3:2/3L:G	95% of maximum N$_{tot}$
Year 1	178 (±25.8)	299 (±37.8)	286 (±31.7)	35
Year 2	173 (±26.2)	211 (±36.4)	253 (±32.6)	24
Year 3	119 (±16.4)	162 (±23.1)	195 (±24.6)	25

b) $_{Ngain}$ (kg ha^{-1} year^{-1})			
	Grass	Legume‡	1/3:2/3 L:G
		N$_{gainleg}$	N$_{gainmix}$ — N$_{gainmix}$/N$_{totmix}$
Year 1	-	121 (±29.7)	108 (±22.7) — 0.38 (±0.090)§
Year 2	-	38 (±24.6)	80 (±18.7) — 0.32 (±0.085)
Year 3	-	43 (±12.1)	76 (±16.2) — 0.39 (±0.097)

869 ‡ Values for pure legume stands (sown as pure legume seeds) are at maximal realized legume
870 proportion across sites, which were 100%, 97%, and 80% in years 1, 2, and 3, respectively
871   (compare Fig. 4).
872 § Approximate standard error of ratio following Lee & Forthofer (2006), p. 37.

873 **Figure legends**

874

875 **Fig. 1.** Sites of the coordinated field experiment to analyze total N yield in grass-legume

876 mixtures. Site numbers refer to Kirwan *et al.* (2014) and are as follows: 1: Belgium,

877   10: Germany_a, 11: Germany_b, 13: Iceland_a, 14: Iceland_b, 15: Ireland, 18: Lithuania_a,

878 20: Lithuania_c, 22: Norway_a, 23: Norway_b, 24: Norway_c, 26: Poland_a, 27: Poland_b,

879 34: Switzerland, 35: Wales_a, 36: Wales_b (see Table S1, Supporting Information, for further

880 information on sites). Symbols refer to the set of plant species sown (see Table 1).

881

882   **Fig. 2.** Illustrative example of potential fractions of total nitrogen (N) yield in grass-legume

883 mixtures and the respective monocultures. In grass monocultures (legume proportion = 0), the

884 total N yield accumulated by the sward derives solely from the soil and from fertilizer N

885 (quantity A). In legume monocultures (legume proportion = 1), the total N yield is greater (A

886 + B), as legumes also have access to atmospheric N through symbiotic N_2 fixation. Mixing

887 grasses with legumes should lead at first to a linear increase of accumulated N by the stand

888 with increasing legume proportion (continuous white line) through a constant amount of N

889 added to the system per unit of legumes. In addition, positive grass-legume interactions (e.g.

890 stimulation of symbiotic N_2 fixation, N transfer) can result in a nonlinear surplus of N yield in

891   mixtures (quantity C). The area of B+C defines the N yield gain ($N_{gain_{mix}}$, see text) for varying

892 legume proportions in mixtures as compared to grass monocultures.

893

894 **Fig. 3.** Total nitrogen yield $_{(Ntot)}$ of monocultures (Grass, Legume) and mixtures at three

895   years for sixteen sites across Europe. N_{tot} is averaged across seed density and additionally

896 across monocultures of the two grass and legume species, respectively. Sites are arranged in

897 order of decreasing means of monoculture N_{tot} averaged over the first two experimental years,

898 with horizontal bars denoting the annual mean N_{tot} of mixtures. Inference on differences

40

899 between mixtures and *grass* monocultures is indicated on top of each panel (Wilcoxon rank

900   sum test on the unpooled data). Missing symbols in b) for site 15 and in c) for sites 15 and 20

901   reflect the absence of legumes. Site numbers follow the codes used in Fig. 1, and missing site-

902 years indicate no analysis of N concentration.

903   *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns = not significant
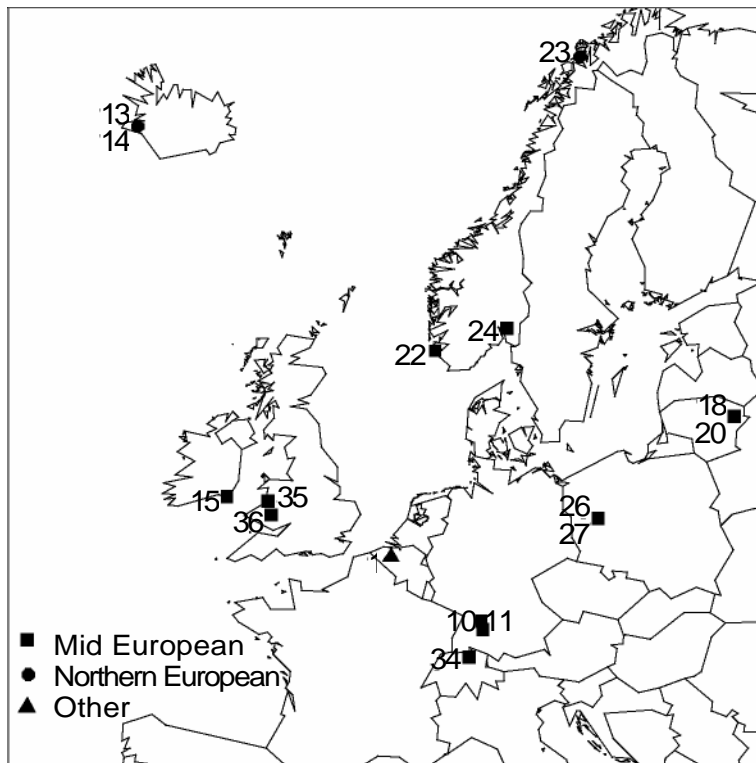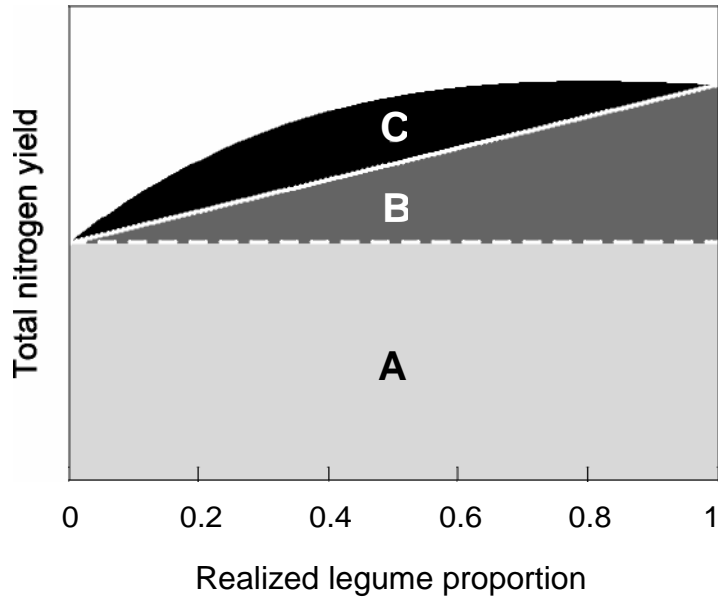
904

905 **Fig. 4** Total nitrogen yield $_{(Ntot)}$ as affected by legume proportion in swards at three years of

906   the multisite grassland experiment. The bold black line displays the predicted $_{Ntot}$ across all

907   sites (fixed mean from linear mixed regression corresponding to Table 2), and coloured lines

908   display predicted $_{Ntot}$ for individual sites (the variation around the fixed mean) over the range

909   of legume proportion realized in the respective year. Numbers on lines refer to sites and are

910 located at the mean legume proportion realized across all stands per site. The shaded area

911   indicates N yield gain in mixture as compared to grass monoculture across sites (compare Fig.

912 2). The horizontal bold line at the bottom of the graph indicates the range of legume

913   proportion for which $_{Ntot}$ across all sites was significantly smaller ($P \leq 0.05$) than at

914 maximum (■); consequently, the dotted line displays the non-significant range. No line could

915 be drawn for site 15 in year two and for sites 15 and 20 in year three because legumes were

916   absent. Other missing site-years indicate no analysis of N concentration.
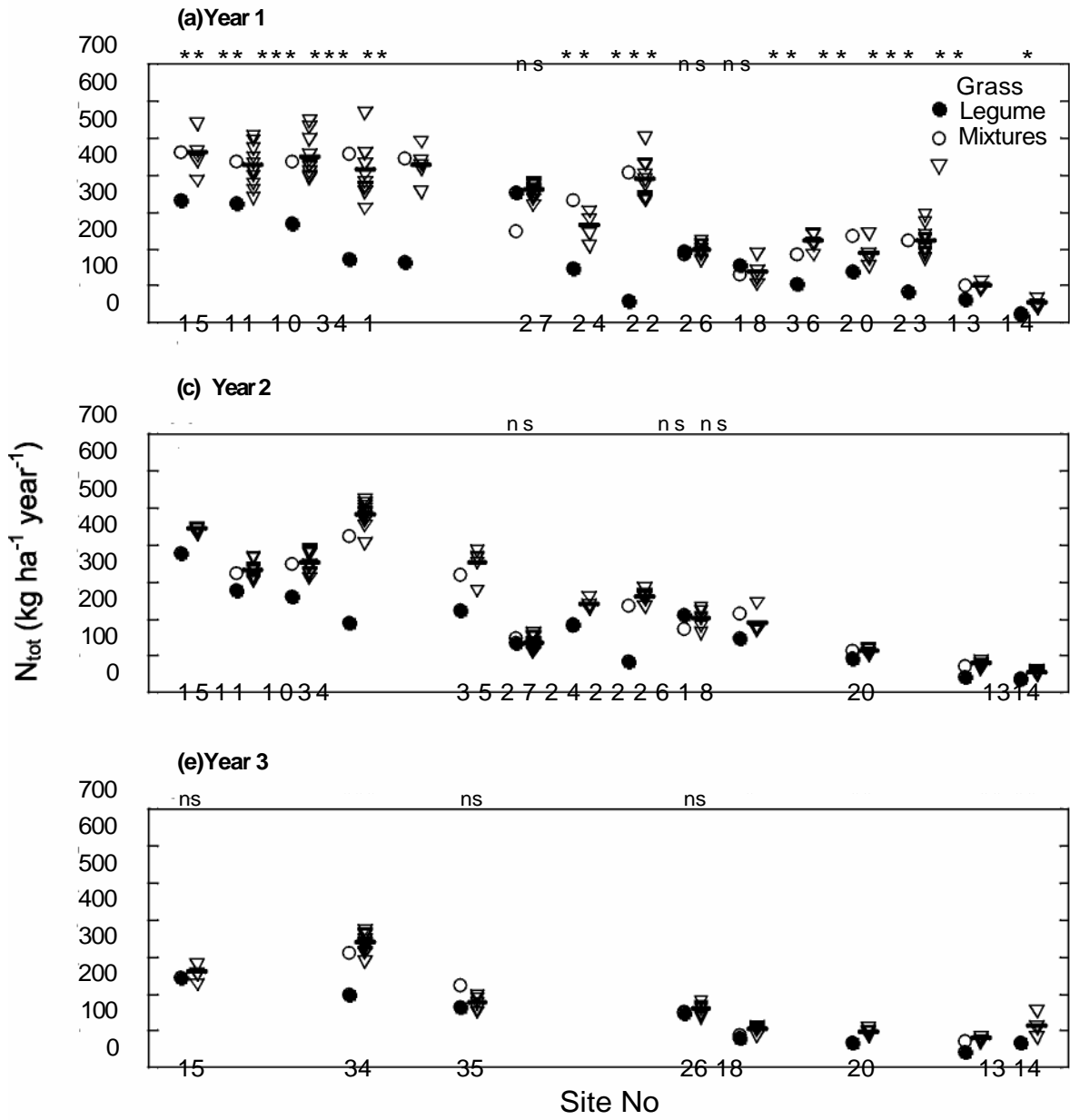
917

918   **Fig. 5.** Correlation between legume proportion in the sward and the climatic variables

919 minimum site temperature (a) and annual precipitation (b), and correlation between the

920   relative N yield gain in mixture $_{(Ngainmix/Ntotmix)}$ and the same climatic variables (c, d),

921   realized legume proportion (e), and site productivity (f). Data are site means across the first

922 two experimental years, with $_{Ngainmix}$ ^{and} $_{Ntotmix}$ being estimated for a mixture with one third

923 proportion of legumes and two thirds of grasses (following eqn. S2, Appendix A, Supporting

924   Information). Pearson's *R*, its significance, and the trend line following least square
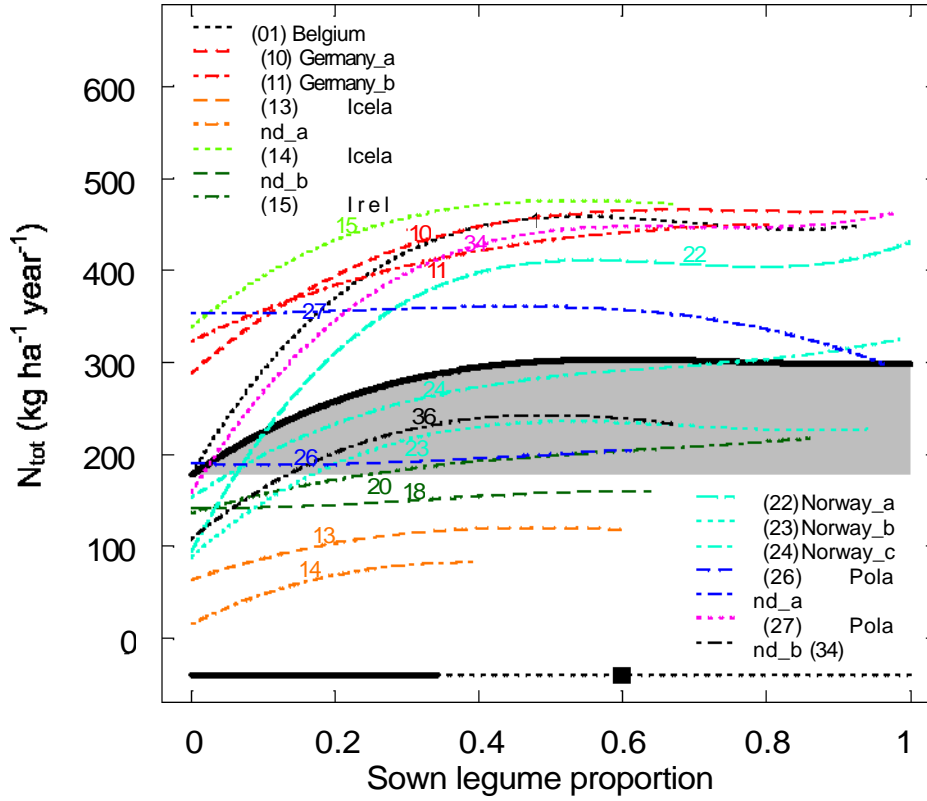
925 regression refer to filled symbols (see Table S7, Supporting Information, for regressions on

926 all climatic variables). Site 15 (Ireland, open symbol) was omitted from these analyses

927 because legumes were absent from the second year onward. For site names to labels see Fig.

928 1.

(a) Year 1

(c) Year 2

N_{tot} (kg ha^{-1} year^{-1})

(e) Year 3

Site No

(a) Year 1

(b) Year 2

(a) $R = 0.703$ $P =$

Realized legume proportion vs Minimum temperature (°C)

(b) $R=0.426$

Realized legume proportion vs Precipitation (mm year^{-1})

(c) $R=0.643$

$N_{gainmix} / N_{totmix}$ vs Minimum temperature (°C)

(d) $R=0.567$

$N_{gainmix} / N_{totmix}$ vs Precipitation (mm year^{-1})

(e) $R = 0.800$ $P <$

$N_{gainmix} / N_{totmix}$ vs Realized legume proportion

(f) $R= - 0.189$

$N_{gainmix} / N_{totmix}$ vs Productivity (t ha^{-1} year$_{-1}$)