Intercropping flowering plants in maize systems increases pollinator diversity

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24 Summary

- 1. Maize is a poorly competitive crop, as such, soil preparation and high 25 application rates of herbicides are required to reduce early competition with 26 27 weeds. This leaves a large amount of bare ground with few flowering weeds, providing a poor farmland habitat for pollinators. 28 2. This study evaluates the effect of four different maize management regimes 29 30 on pollinator diversity and community composition. 3. Flowering plants intercropped with maize attracted pollinators, helping to 31 32 support pollinator communities. Similar intercropping techniques using a grass ground cover did not increase pollinator density, demonstrating that pollinator 33 richness, density and diversity is intrinsically linked to the presence of 34 35 flowering plants. 4. A maize system with a diverse intercrop may make it possible for pollinators 36 to thrive, however, these systems may only be attractive enough to bring 37 pollinators in temporarily from the surrounding areas. 38 **Synthesis** 39 5. These results show that there can be significant improvements to pollinator 40 diversity, density and community composition through modifying maize 41 cultivation practices, however, these benefits must be balanced with yield 42 43 penalties of ca. 60% to farmers. 44 Keywords 45 Agro-ecosystems, bee, community, density, diversity, maize, richness,
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48 **1. Introduction**

Arable production, especially maize (Zea mays L.), adversely affects pollinator 49 biodiversity (Geiger et al., 2010; Carvell et al., 2011), which is in decline globally 50 (Biesmeijer et al., 2006; Carvell et al., 2011; Goulson, 2015). Whilst there is a need 51 to produce versatile crops, such as maize, to meet agricultural demands (Edgerton, 52 2009), these must be balanced with protecting ecosystem services, including those 53 that are facilitated by biodiversity (DeFries et al., 2004; Wodika & Baer, 2015) such 54 as pollination (Delaplane et al., 2000). Pollinator biodiversity depends on four main 55 56 characteristics of an agro-ecosystem: the diversity of vegetation within and around the system, the permanence of the various crops within the system, the intensity of 57 management, and isolation of the system from natural vegetation (Altieri, 1999; 58 59 Holzschuh et al., 2007; Batary et al., 2010).

Maize is an increasingly important, multifunctional crop with over 184,000 ha 60 grown annually in the UK (DEFRA, 2015) being used as forage for cattle and is 61 becoming increasingly important as a feed stock for biogas generation 62 (Hochholdinger and Tuberosa, 2009; Adams, 1989; Banse et al., 2008). However, 63 Maize is a poorly competitive crop; therefore intensive soil preparation and high 64 application rates of herbicide are required to reduce early competition with weeds 65 (Hall et al., 1992). This leaves a large amount of bare ground, providing poor habitat 66 67 for biodiversity, especially for pollinators (Carvell et al., 2011; Hawes et al., 2009; Potts et al., 2006; Barbir et al., 2015). Wilson et al. (1999) identified that these 68 reductions in the diversity and abundance of food plants leads to reduced 69 70 invertebrate diversity, which is a result of the combination of frequent tillage, improved seed-cleaning technologies and herbicidal weed control. Unfortunately, 71

conventional maize cultivation fulfils the criteria set out by Wilson *et al.* (1999) to
erode pollinator biodiversity.

Novel maize cultivation techniques such as sowing the crop into an 74 understorey of legume mixes or grasses have been shown to reduce negative 75 environmental impacts such as nutrient and soil loss by promoting rainfall infiltration, 76 improving soil stability and reducing run-off (Hartwig & Ammon, 2002; Manevski et 77 al., 2015). These techniques have also been shown to support the farmland 78 biodiversity by providing resources for many species (Pywell et al., 2005; Norris et 79 80 al., 2016). However, because maize is a poor competitor in its early stages of growth it is easily outcompeted by intercrops. As such, intercrops must be controlled to 81 maintain maize yield (Nakamoto & Tsukamoto, 2006). Mowing, grazing or spraying 82 83 with a herbicide have all been shown to maintain crop yield similar to that found in conventional cropping systems, whilst maintaining the above benefits (Nakamoto & 84 Tsukamoto, 2006). However, contrasting studies have shown that even with control 85 of intercrops by mowing, maize yields can be significantly reduced due to root 86 system competition (Liedgens et al., 2004; Norris et al., 2016). 87

Supporting pollinator diversity in arable systems is currently a hot topic 88 (Goulson, 2015). Colonisation by invertebrates is generally dependent on natural 89 dispersal from the regional species pool (Chateil, 2015), which can be highly 90 91 fragmented (Hilderbrand et al., 2005), and where there are greater numbers of flowering plant species there will be greater richness, density and diversity of 92 pollinators. In arable systems, a disturbance such as ploughing or tillage disrupts 93 94 this colonisation process, and although biotic and abiotic conditions can be restored to these systems, community assemblages sometimes cannot (Hilderbrand et al., 95 2005). Despite this, recent work by Wodika & Baer (2015) has shown that this is not 96

necessarily the case, even with soil fauna, which have lesser dispersal efficiency 97 compared with pollinators (Giller, 1996). Wodika & Baer (2015) showed that after 98 disturbance there can be a recolonisation by taxa comprising of a similar community 99 100 assemblage to the pre-disturbance state, if the vegetative community is restored. Sadly, in conventional maize cultivation systems, there are often few flowering non-101 crop plants, which limits forage resources for pollinators (Hawes et al., 2009; Potts et 102 al., 2006). As maize is wind pollinated, increasing pollinators in maize systems would 103 not directly benefit the maize crop, however, supporting pollinators at the farm or 104 105 landscape scale would benfit other crops such as Brassica napus L. as well as wild flora. 106

In this study, we investigated the effect of four contrasting maize cultivation and ground cover management practices on pollinator density, diversity and community composition and maize yields, to identify sustainable maize production techniques. We hypothesized that where greater numbers of flowering plants were sown in the strip tillage biodiverse seed mix cultivation methods, larger populations of pollinators would be encountered.

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114 **2. Materials and methods**

115 **2.1. Site description**

Field experiments were established in a conventionally ploughed maize crop at two study sites over two cultivation seasons in April 2013; the first site was in the South West of England near Bow, Devon and the second in the east of the UK, near Fakenham, Norfolk. The study sites were selected for the freely draining, slightly acidic loam soil at Bow and freely draining slightly acid but base-rich soil at Fakenham (Driessen, 2001), which is typical of land under maize cultivation in the

UK. Maize is often grown in the same field year after year in the UK, as such the 122 intercrops were established in 2013 and the maize in the subsequent two cropping 123 seasons, drilled into the rows that the previous year's crop occupied. Twelve study 124 plots at each site, 10 m wide and 60 m in length, with a 1 m gap between plots, were 125 established in a randomised replicated block design with three replicates of each 126 treatment. Four contrasting maize cultivation techniques were selected to test the 127 effects on pollinator diversity and community structure: 1) conventional plough and 128 subsoiled (PGH), 2) minimum tillage (MNT), 3) strip tillage into perennial ryegrass 129 that was sown at a rate of 35kg ha⁻¹ (RGS) in June 2012, and 4) strip tillage into a 130 biodiverse seed mix (BSM). The BSM strip crop was over sown with a commercially 131 available seed mix in June 2012 at 15 kg/ha⁻¹ containing Medicago lupulina L. 20%, 132 Onobrychis viciifolia L. 25%, Trifolium hybridum L. 20% Trifolium incarnatum L. 133 subsp. Incarnatum 20%, Lotus corniculatus L. 10%, Malva moschata L. 5%. For a 134 full experimental design and agronomic details please refer to Norris et al., (2016). At 135 Fakenham in 2013, pre-emergence application of herbicides were 4.5 I ha-1 Stomp® 136 (a.i Pendimethalin) to all treatments; 150ml ha-1 Reglone® (a.i Diguat) to the two 137 strip tillage treatments and 3.5 I ha-1 of Hoedown® (a.i Glyphosate) was applied to 138 the ryegrass treatment. Post-emergence application of herbicides were 1 I ha-1 139 Touchdown® (a.i Glyphosate) applied to the ryegrass plots; Callisto® (a.i 140 141 Mesotrione) at a rate of 1 I ha-1 was applied to PGH and RGS and to the BSM treatment at a rate of 0.5 I ha-1. At Bow in 2013, Pendimethalin (Stomp ©) was 142 applied to all plots except the BSM treatment at application rate of 3.0 l ha-1, with an 143 144 additional 1.0 I ha-1 Samson applied to the perennial ryegrass plots. At Fakenham, in 2014, to reduce intercrop competition and improve yields additional herbicides 145 were applied to the strip-tillage treatments compared with 2013; 5 I ha-1 Wing P® 146

(a.i Pendimethalin) was applied to all treatments. Touchdown® at 1 I ha-1 (a.i 147 Glyphosate) was applied to all treatments except BSM where Touchdown® was 148 applied at a half rate of 0.5 I ha-1. Post-emergence, Callisto® was applied at a rate 149 of 2 I ha-1 to all treatments. At Bow, in 2014 Gallup® was applied to the BSM 150 treatment at a rate of 1.5 I ha-1, Non-inversion at a rate of 3.0 I ha-1, Conventional at 151 a rate of 3.0 | ha-1 and Ryegrass at a rate of 0.75 | ha-1. After the maize was sown 152 at Devon Wing-P® was applied at a rate of 4 I ha-1 with an additional later spraying 153 of Calaris® at 1 I ha-1 and Samson at 0.5 I ha-1 to reduce competition from the 154 155 existing plant cover.

At both sites 150 kg N ha-1 of ammonium nitrate (a.i nitrogen) was applied to all treatments in 2013. At Fakenham in 2014 potash (175 kg ha-1) was applied to all treatments. At Bow in 2014 100kg N ha-1 in the form of Urea and 20 kg N ha-1 in the form of monoammonium phosphate (MAP) was applied to all cultivation methods, and at Norfolk N-fertiliser applications remained the same as in 2013.

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162 **2.2. Pollinator survey**

Pollinators were counted using the line-transect method developed for the UK 163 butterfly-monitoring scheme (Pollard & Yates, 1993), which has been adapted as a 164 standard method for bee surveys (Roy et al., 2003; Banaszak, 1980). Pollinator 165 166 transects were walked on a weekly basis during June and July in both field trial years, coinciding with the legume flowering period. Pollinator transects were carried 167 out by walking 60 m along the intercrop area, but excluding the outermost 1 m of 168 169 each plot. Surveyors used transect recording sheets to score the pollinators foraging on each plot. Given the need to identify bees while on the wing, counts were made 170 for groups of bumble-bee (Bombus) species based on colour type, and Apis were 171

also noted (Prys-Jones and Corbet, 1991). Walks were performed between 10.00 172 and 17.30 when weather conformed to UK butterfly monitoring scheme standards 173 (wind speed less than 5.5 m s⁻¹, not raining, temperature greater than 17 °C if sky 174 overcast or greater than 13 °C if sky at least 60% clear) (Pollard & Yates, 1993; Roy 175 et al., 2003). The order in which transects were walked was randomised (Roy et al., 176 2003; Haughton et al., 2003), using a random number generator. The time taken for 177 each transect depended on crop stage, but observers walked at approximately 12 m 178 per minute (Banaszak, 1980; Roy et al., 2003; Westphal et al., 2007). 179

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181 **2.3. Vegetation survey**

Visual assessments of vegetation were carried out in late July in 2013 and 182 183 2014. Six vegetation samples were located within the inter-row areas of each plot with a rectangular quadrat (1.0 m x 0.25 m) placed at different locations, parallel to 184 the rows of maize. Percentage cover by vegetation, litter and bare ground, together 185 with vegetation richness (number of plants 0.25 m²) were recorded for each plot. At 186 Fakenham, a conventional maize harvester was used to measure crop yield, with the 187 harvested area of each plot being 7.5 m x c.60 m. At Bow, maize yields were 188 measured from each plot from a 10 m by 2 m area, and each maize plant within the 189 demarcated area was harvested by cutting approximately 15 cm above the soil 190 191 surface. Maize plants from both sites were chopped using secateurs before analysis. Harvested samples were oven dried at 60 °C for 24 hours and the dry 192 weight recorded. Dry weights were multiplied to give a total dry matter per plot and 193 194 are reported on a tons per hectare basis.

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196 **2.4. Statistical analysis**

All statistical analyses were conducted using R v3.0.1 (R Core Team, 2013). The 197 plant community measurements and total numbers of pollinators observed over six 198 sampling weeks in each sampling year were used to test for differences in richness, 199 200 density and Shannon diversity using analysis of variance on Box-Cox transformed data (Højsgaard 2006). Density was expressed as the total number of pollinators 201 observed over the six transect walks preformed at each site in 2013 and 2014, with 202 203 field site being used as a random effect. Once data were normalised, Tukey HSD tests were used to test for significant differences between cultivation methods and 204 205 field trial years. The diversity for each of the cultivation methods was determined using the Shannon-Weiner diversity index (H) calculated using the R package 206 'vegan' (Oksanen et al., 2007; Norris et al., 2016). 207

208 Pollinator community counts were Wisconsin square root transformed using Euclidian distances among sites and years to measure species that were associated 209 with changes in vegetation, using two-dimensional non-metric multidimensional 210 scaling (NMDS) which was selected using stress vs. dimensionality to identify the 211 number of axes that best represented the community. The vegetative variables and 212 plant species count data were fitted to the NMDS species scores using the function 213 'envfit' (Oksanen et al., 2007) based on 999 permutations. Vegetative variates and 214 plant species with a significant influence on pollinator community composition (P < P215 216 0.05) were plotted based on the results from 'envfit'. As pollinators were predominantly observed in BSM plots (Table 1), further statistical analysis was 217 performed by separating this cultivation method from the other three cultivation 218 219 methods and assessing the influence plant species composition had on pollinator communities. 220

222 **3. Results**

The richness, density, and diversity of pollinator communities were 223 significantly greater in the strip tillage into a biodiverse seed mix cultivation method 224 compared to the other three cultivation methods (Table 1). Although some pollinators 225 were encountered in the other cultivation methods, a majority of pollinators were 226 observed in the strip tillage into a biodiverse seed mix cultivation method (Table 2). 227 228 In 2014, the increase in herbicide application reduced the richness of vegetation in the biodiverse seed mix cultivation method (Table 1 and Table S1). 229 230 However, the overall richness, density, and diversity of the pollinator community was not significantly reduced (Table 1). 231 B. lapidarius and B. terrrestris/lucorum were the most frequently observed, 232 233 predominately found foraging in the strip tillage into a biodiverse seed mix cultivation

method (Table 2). Despite the increased application of herbicides in 2014, and the subsequent reduction in plant richness (Table 1) the number of *B. hortorum* observed increased (Table 2, P = 0.008). However, the abundance of *B. pascuorum* was significantly reduced in 2014 (P = 0.008).

Plant communities were composed of different species in the different treatments (P < 0.001), which explained 50% of the variation (Table 3). Community composition between the two sites was significantly different (P < 0.001), although these differences only explained 11% of the variation in overall pollinator composition (Table 3).

Vegetation richness had a significant influence (P < 0.001) on pollinator community composition, explaining 32% of the overall variation (Table 3). Bare ground, negatively correlated with vegetation cover, had a significant influencing effect on community composition (P < 0.001) explaining 15% and 26% of the variation respectively (Table 3).

Separating the BSM pollinator community for more detailed analysis showed that field site and sampling year had a significant effect (P < 0.001) on pollinator community composition, which explained 88% of the variation (Table 3, Fig. 1). Vegetation cover also explained a significant (P = 0.044) amount of variation (49%) in pollinator community composition (Table 3, Fig. 1).

Plant species composition also influenced the composition of pollinators ($F^{1,10}$ 253 =7.54, P =0.020, slope = 15.29), Fig 1). The plants that were sown in the BSM 254 cultivation method established well, however the plant species that influenced 255 difference in composition between plots and sites were the opportunistic weed 256 257 species that re-emerged after ploughing (Norris et al., 2016). Urtica urens L. correlated with greater observed numbers of Apis. (Table 6, Fig 1). In contrast, 258 Epilobium ciliatum Raf., Matricaria recutita L., Senecio vulgaris L. and Trifolium 259 repens L. were found to be associated with greater observed numbers of B. 260 terrrestris/lucorum and B. pratorum (Fig 1). 261

Although cultivation year did not significantly effect (P = 0.775) community 262 composition (Table 3) when considering both sites and all cultivation methods, there 263 were significant interaction differences in community composition associated with the 264 265 two sites which changed between the two years in the strip tillage into a biodiverse seed mix cultivation method (Fig. 1, $r^2 = 0.88$, P = 0.001). B.hortorum was associated 266 with Bow in 2014, however, in 2013 the community observed at the Bow site was 267 more associated with greater densities of B. terrrestris/lucorum (Fig. 1). B. 268 pascuorum and B. lapidarius were associated with the Fakenham site in 2014. 269

Unlike at Bow, there was no significant difference in community compositionbetween the two years at Fakenham (Fig. 1).

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273 **4. Discussion**

Understanding how to manipulate agrosystems to promote ecosystem services such as pollination is a key goal of agro-ecology (Altieri, 1999). This study has shown that a flowering plant ground cover is important for supporting pollinators. There is strong evidence to suggest that having a number of flowering plants in the biodiverse seed mix ground cover significantly increased the richness, density and diversity of pollinators. (Table 1 and Table S1).

Maize in the UK is often grown year after year in the same field. As such, the 280 281 experiment mimics conventional practice by performing the field trails for two consecutive years in the same fields using the previous year's maize crops as a 282 guide for where to drill the subsequent crop. Although the maize yield was reduced 283 284 in the two strip tillage cultivation methods compared with the conventional and minimum tillage cultivation methods (Table 1), in the second year early competition 285 of the intercrops with maize was reduced, increasing maize yields without 286 significantly reducing the richness, density or diversity of pollinators (Table 1). This 287 result confirms that flowering plant availability is a key driver of pollinator abundance 288 289 and diversity. At the landscape scale, increasing the density of flowering species within arable systems could provide greater resource complexity and stratification to 290 help support pollination and pollinator communities (Roy et al., 2003; Potts et al., 291 2006). 292

Separate analysis of the BSM cultivation technique showed that vegetation
 richness and cover are positively correlated under BSM and vegetation cover was a

key factor influencing the composition of pollinators utilising these resources (Table
3) suggesting that where there was a greater cover and richness there was greater
variety of plant species for pollinators to forage upon.

The separate analysis of the data from the BSM plots (Table 3) showed that 298 the composition of the pollinator community differed between the two sites. Others 299 studies have shown that this may be attributed to natural variation in the surrounding 300 local pollinator populations from which communities were able to recruit (Baur et al., 301 1996; Tsiafouli et al., 2015). Although not measured in this study, differences 302 303 between communities over large spatial scales have been shown to be linked to the surrounding vegetation and the extent of isolation from natural vegetation (Altieri, 304 1999; Roy et al., 2003). Despite the differences in community composition at the two 305 306 sites (Table 3), greater richness, density and diversity of pollinators was associated with an increase in the cover of flowering plants (Table 1). Of all the observed 307 pollinators, Apis was found to be particularly sensitive to increases in vegetation 308 cover, which correlated with greater numbers of U. urens (Fig 1). In contrast, B. 309 terrrestris/lucorum abundance was correlated with greater numbers of T. repens (Fig. 310 1). These results suggest that different pollinators may be associated with different 311 plant species, altering foraging strategies among pollinator species; supported by 312 evidence of site choice by pollinators being related to the number, size, colour, scent 313 314 of flowers present (Schlinkert et al., 2015) as well as morphological traits of pollinator species such as tongue length (Hegland et al., 2005). 315

These results show that it is possible to support greater pollinator populations than are currently supported under conventional maize systems by including only a few species of flowering plants (Table 3). However, plots with greater cover of flowering plants supported more diverse pollinator communities (Fig 1; Schlinkert *et al.*, 2015).

Although this study shows that it is possible to increase the biodiversity of 321 pollinators in maize systems by increasing the number of flowering plants (Goulson, 322 2015), these benefits to pollinators must be balanced with the yield penalties to 323 farmers. At both sites, the greatest dry matter yields were measured on the 324 325 conventional and non-inversion cultivation methods, with a mean dry matter yield of c.11 t/ha. However, yields from the strip tillage-ryegrass (RGS) and strip tillage-326 327 biodiverse mix (BSM) were as much as 80% and 90% lower than the conventional treatment at Norfolk and Devon respectively (Table 1). Increased herbicide 328 application rate to the intercrop in 2014 reduced early competition with maize and 329 330 improved yields (Table 1), however, yields on the strip tillage-ryegrass and strip tillage-biodiverse mix were still approximately 45% lower compared to the 331 conventional treatment. Despite reductions in yield, this study suggests that even a 332 relatively low richness of flowering plants will attract pollinators, supporting 333 populations and the services they facilitate. Despite increases in pollinator richness 334 and diversity a maize system with diverse intercrops may make it possible for 335 pollinators to thrive, however, these systems may only be attractive enough to bring 336 pollinators in temporarily from the surrounding areas. Agricultural schemes to secure 337 338 the important ecosystem services such as pollination are critically important for sustainable crop production. Further work should focus on investigating the 339 proportions of the cultivated area that needs to be sown with flowering plants to 340 support greater numbers of pollinators in arable systems, how this affects pollinator 341 diversity on a landscape scale and the longevity of these effects. 342

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486 **Abbreviations**

- 487 Conventional plough-based maize cultivation (PGH), minimum tillage maize
- 488 cultivation (MNT), strip tillage into a biodiverse seed mix ground cover (BSM), strip

- tillage into a perennial ryegrass ground cover (RGS), Non-metric multidimensional
- 490 scaling (NMDS).