

The impact of sunflower crop management on the conservation of foraging bumblebee (*Bombus spp.*) communities

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1. Abstract

A rapid decline of 33% of wild pollinating species since 1980 in the UK has meant management strategies within agroecosystems for conserving pollination services is increasing in importance. Recent interest has occurred in utilising mass flowering crops (MFCs), such as sunflowers (*Helianthus*), as a pollinator resource. However, research addressing specific pollinator communities within sunflower crop management and how these communities affect ecological functioning, are scarce. The present study investigates how sunflower crop management impacts bumblebee (*Bombus*) communities and their ecosystem services, thereby allowing the creation of effective, evidence-based management objectives. Foraging bumblebee communities were surveyed in the incrop and outcrop (2 m wide wildflower belt) of three sunflower fields at Rhossili, South Wales, and a control NNR site, Oxwich. Their ecosystem service role was evaluated through analysing functional trait diversity and individual-level variation using pre-selected bumblebee traits (tongue length, ITD mean measurements, foraging range, sex and flower visitation length). Bumblebee diversity was higher within the sunflower incrop than the wildflower outcrop ($F_{(1,4)} = 9.464, p = 0.022$) and community composition differed between these habitats with species such as *Bombus lapidarius* and *Bombus terrestris* primarily foraging within the sunflowers. Yet, individual-level variation and all functional trait diversities, apart from sex, did not vary. These results suggest that MFCs do benefit wild bumblebee communities, likely due to the provision of additional large foraging resources, thus highlighting sunflowers' potential within bumblebee conservation. The outcrop margin is still considered essential in encouraging agroecosystem resilience as it maintains board bumblebee community compositions and foraging opportunities when the crop is not flowering. However, the lack of variation in functional trait diversity across the habitats implies that management involving sunflowers may have little impact on the conservation of their ecosystem service role. This, alongside the possible potential for MFCs to compete with adjacent wildflowers and cause pollinator dilution, highlights that more research into pollinators' functional diversity and MFCs' limitations are needed before MFC is implemented as a pollination services conservation strategy.

Keywords: Pollination services, Conservation, Bumblebee, Functional diversity, Mass flowering crops, Sunflowers

2. Introduction

Pollination is an ecosystem service that plays a critical ecological and societal role, regulating crops and wild plant communities; helping ecosystems to be more resilient to disturbance (Ollerton *et al.*, 2011; Vanbergen & Initiative, 2013). However, this ecosystem service is threatened by global population declines in wild pollinating insects (NRC, 2007; Williams & Osborne, 2009; Bommarco *et al.*, 2011), with 33% of wild species within the UK having decreased since 1980 (Powney *et al.*, 2019). Anthropogenic global change pressures such as the rise in agricultural intensification and landscape alterations have been identified as potential causes for these declines (Tschamntke *et al.*, 2005; Brown & Paxton, 2009; González-Varo *et al.*, 2013). This, alongside the decline in domestic pollinators such as *Apis mellifera*, has resulted in a “global pollinator crisis” (Aizen & Harder, 2009; Levy, 2011; Shivanna, 2019).

To address the current pollinator crisis and the key drivers behind the pollinator declines, management schemes, such as the European Union led agri-environmental schemes (AES), have been researched and implemented (Scheper *et al.*, 2013; Kovács-Hostyánszki *et al.*, 2017; Ouvrard, Transon & Jacquemart, 2018). The focus of these schemes is to conserve a diverse range of wild pollinators over singular domestic pollinators, as functional life history differences within wild communities allow for varied responses to potential environmental change and greater functionality within ecosystems (Hoehn *et al.*, 2008; Garibaldi *et al.*, 2013). These management schemes include within crop action, such as the utilisation of organic farming (Hillocks, 2012), along with outcrop measures such as placing wildflower strips around agricultural fields (Feltham *et al.*, 2015) or setting aside fields sown with suitable wild plants (Alanen *et al.*, 2011; Blaauw & Isaacs, 2014). Although these management schemes have been found to enhance pollinator diversity at local and landscape scales (Jonsson *et al.*, 2015), their success at national scales is dependent on farmer uptake, which is still low within the UK (Baude *et al.*, 2016; Senapathi *et al.*, 2017). Therefore, to encourage farmer participation, recent investigation has been undertaken into utilising mass-flowering crops (MFCs), such as rapeseed, *Brassica napus* or sunflowers, *Helianthus annuus*, within these schemes (Westphal *et al.*, 2003; Todd, Gardiner & Lindquist, 2016).

The farming of sunflowers as non-native MFCs within agricultural landscapes are known to increase wild bee abundances as the flowers provide large resources of nectar and pollen (Jones &

Gillett, 2005; Hoehn *et al.*, 2008). Yet, obtaining a larger abundance does not always determine a successful management scheme as it ignores the pollinator's ecological functionality (Knight *et al.*, 2005; Forrest *et al.*, 2015). Regardless of this knowledge, studies addressing specific pollinator communities within sunflower crop management and how they impact ecological functioning, are currently scarce (Geslin *et al.*, 2016). Evaluating the diversity and composition of specific functional traits, classified by species or by individual (Roches *et al.*, 2018), is a possible way to address this (Cadotte, Carscadden & Mirotnick, 2011). Functional trait diversity is an approach that is rapidly gaining interest and importance within animal community ecology, and although the exact definition varies (Petchey & Gaston, 2006; Ricotta & Moretti, 2011; Wood *et al.*, 2015), it is referred to within this study as the evenness and relative abundance of species' life-history traits which influence ecosystem functioning (Petchey & Gaston, 2006; Wood *et al.*, 2015).

Bumblebees, *Bombus spp*, the pollinator community focused on within this study, are efficient wild pollinators within semi-natural and agricultural habitats (Corbet, Williams & Osborne, 1991). Todd, Gardiner & Lindquist (2016) highlight that a sunflower crop can support bumblebee communities, and suggest that the pollinator communities could be broader when utilising the MFC alongside other conservation management strategies. However, the study does recognise more research is needed to verify these suggestions and apply them to bumblebee conservation schemes.

Building on Todd, Gardiner & Lindquist (2016), this study aims to determine how sunflower crop management affects foraging *Bombus* communities and to relate this to their ecosystem service role through functional trait metrics and individual-level variation. This would thereby provide clear future management objectives to ensure the security of wild *Bombus* pollination services, subsequently enhancing ecosystem resilience. Thus, this study hypothesises that, depending on sunflower density and outcrop composition, in comparison to wildflower outcrops and controls, i) the bumblebee diversity is greater within the sunflower crops, ii) the bumblebee community composition differs between the habitats, and iii) bumblebee functional trait diversity and visitation length per flower is higher within the sunflower crops.

3. Methods

3.1 Study sites

The study was primarily conducted in the Vile, Rhossili, South Wales (Figure 1, Table 1). The Vile is on the coastline and is characterised by a patchwork of different sized crop fields interwoven with wide hedgerows. This site provided the opportunity to test whether the agroecosystem methods introduced by the National Trust, which utilise *Helianthus annuus*, have impacted foraging Bumblebee populations. As there were no wildflower fields at the Vile, a wildflower meadow at Oxwich was selected as the control site (Table 1). This site was also coastal and was a semi-natural, open, wildflower meadow with a grassy ground layer. Three organically grown (50 m x 200 m) sunflower fields at the primary site and the wild meadow at the control site were studied from the 11th - 27th August 2019. Each field had a two-metre wide human-defined wildflower outcrop, without *Helianthus annuus*, bordering the field and an understory of the same wildflowers throughout the crop. Each field's wildflower outcrop was abundant in *Trifolium pratense* or *Cirsium arvense* and the incrop had either a low or high density of sunflowers (Table 1).

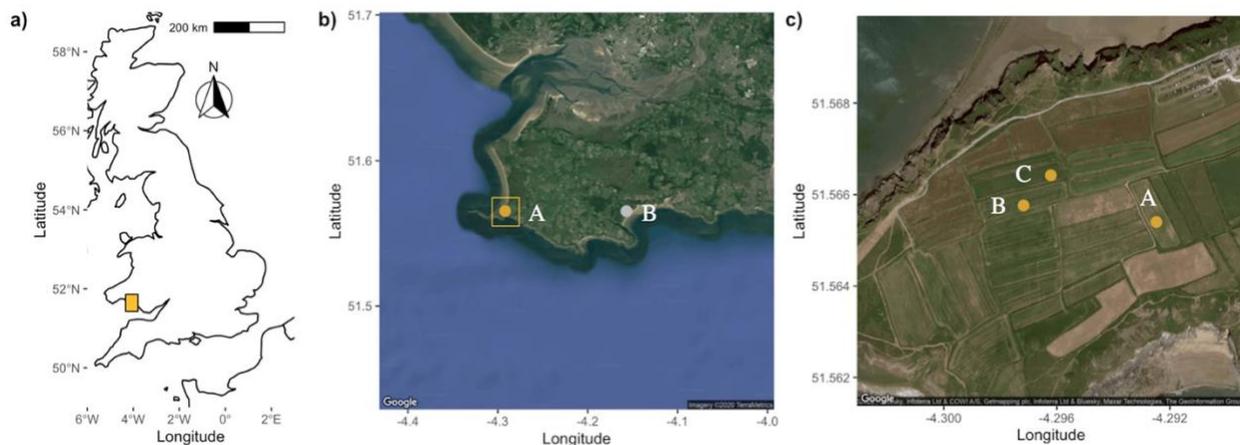


Figure 1: Study site distribution. a) South Wales location of study sites within the UK, b) Data collection locations within South Wales: A. primary site, Vile, Rhossili, B. control site, Oxwich, c) Three sunflower fields within the primary site: Field A, Field B, Field C. (Kahle & Wickham, 2013).

Table 1: Description of the control and areas within each sunflower field at Rhossili and the external conditions (temperature and time of day) when data collection occurred stated.

Field / Site	Latitude, Longitude	Sunflower density	Main outcrop flowering species	Incrop / outcrop	Temp. range/°C	Time of day tested
A	N 51 56 54 01, E -4 29 24 75	Low	<i>Trifolium pratense</i>	Incrop	18.1 - 27.2	Morning, Midday, Afternoon
				Outcrop	19.8 - 26.1	Morning, Midday, Afternoon
B	N 51 56 57 62, E -4 29 71 75	High	<i>Trifolium pratense</i>	Incrop	15.0 - 23.7	Morning, Midday, Afternoon
				Outcrop	18.0 - 22.3	Morning, Midday, Afternoon
C	N 51 56 64 23, E -4 29 62 13	High	<i>Cirsium arvense</i>	Incrop	17 - 25. 6	Morning, Midday, Afternoon
				Outcrop	17.5 - 23.6	Morning, Midday, Afternoon
Oxwich	N 51 56 53 02, E -4 15 68 91	None	<i>Chamerion angustifolium</i> & <i>Oenothera glazioviano</i>	Control	19.7 - 25.1	Morning, Midday, Afternoon

3.2 Sampling methods

Bombus individuals were sampled within a total of forty 15 m transects. On the primary study site, 34 transects were undertaken with six transects in the outcrop and incrop of each sunflower field, discarding one outcrop and incrop transect from sunflower field C due to tourist caused destruction. Each incrop transect was paired with an outcrop transect to allow for a direct and standardised comparison (Figure 2). On the control site, six 15 m unpaired transects were used. Transects were selected randomly around the borders of the fields and the control site, though at least 15 m apart. All transects were undertaken under similar climatic and environmental conditions; between 08:00 and 18:00 on days with temperatures above 12 °C; dry with minimal dark clouds; and wind speeds below 4.5 m/s (Table 1; Hoehn *et al.*, 2008; Dupont, Damgaard & Simonsen, 2011).

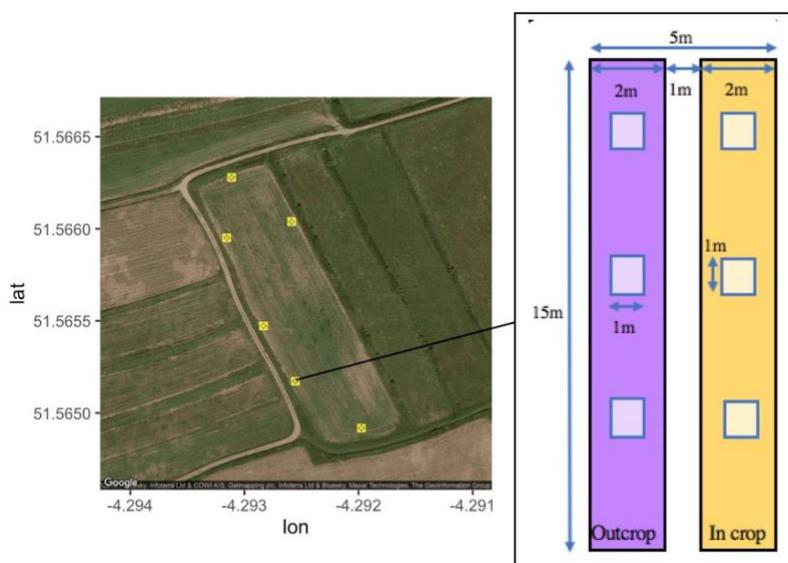


Figure 2: The experimental design of one of the paired transects. Outcrop = field edge (no sunflowers and hedgerows). Incrop = one-metre into sunflower crop. The visitation length surveys and flower surveys (shown in Appendix) occurred in each 1 m x 1 m plot.

Firstly, 10-minute observations were conducted within three 1 m x 1 m quadrats spaced 5 m along the 15 m transects (Figure 2). A habituation period of one minute was executed before each observation period began to reduce the observer effect (Wade, Zalucki & Franzmann, 2005). The bumblebee individuals which travelled into the marked patches were identified to species level, apart from *B. pascuorum*, *B. humilis* and *B. muscorum* which were labelled as their subgenus *Thoracobombus* and *B. lucorum* and *B. terrestris* which were labelled as *B. terrestris*, the more commonly found species in the study site. The sex, time spent on the first flower, and the flower species landed on was recorded.

After these observations, a timed transect walk was undertaken within the same transect. *Bombus* individuals occurring on the anthers or stigmas of flowers 1 m either side of the transects were collected using an aerial net. Individuals were not released until the end of transects to avoid possible re-recordings. To standardise this method and reduce observer bias, the timer was paused during the *Bombus* captures. For each individual collected, the species and sex were recorded in the field, noting that worker females *B. terrestris* and *B. lucorum* were not distinguished between as it is unreliable in the field (Goulson & Darvill, 2004). As all of the individuals could not be collected by this sampling method, the sampling error was noted (Appendix 1).

3.3 Functional traits and diversity indices

Trait selection for this study was based on *Bombus* specific characteristics which are recognised to vary across the taxa and relate to their role in pollination (Table 2, Fontaine *et al.*, 2006; Moretti *et al.*, 2009). To address the species level variation, four species and sex-specific functional traits were selected: tongue length (mm), intertegular distance (ITD) mean measurement, foraging range, and sex. To address individual-level variation, a behavioural trait was used (visitation length). Secondary data on ITD and tongue length for all species, apart from male *B. muscorum* and *B. jonellus*, were obtained from XXX.

Table 2: The trait groups for each ecological level used in the study, their definition and selection rationale.

Trait groups	Variables	Definition / description	Selection rationale
Species level			
Sex	Binary, coded a) Female (1), b) Male (0)	a) Bumblebee which started as a fertilized egg and has pollen baskets or corbiculae on metatibial. b) Bumblebees which started as unfertilised eggs and do not have pollen baskets.	Key variation in behaviour and mode of pollen transport. a) Eusocial, pollen collected by metatibial corbiculae (Wappler <i>et al.</i> , 2015). b) Live outside nest, pollen transported accidentally by body hairs (Goulson, 2010).
ITD mean measurement	Semi-quantitative Scaled: 0=lowest, 1=highest	Distance between nearest edge of the plates (tegulae) which are covering the wing bases (Williams <i>et al.</i> , 2010)	Considered a proxy for foraging range (Greenleaf <i>et al.</i> , 2007) and dry body mass or size (Cane, 1987; Hagen & Dupont, 2013). A well-known relationship with bee foraging behaviour (Greenleaf <i>et al.</i> , 2007; De Palma <i>et al.</i> , 2015).
Foraging range	Calculated using allometric conversions on ITD (Greenleaf <i>et al.</i> , 2007)	Maximum distance from nest at which foraging is still energetically profitable (Nieuwstadt & Iraheta, 1996; Greenleaf <i>et al.</i> , 2007). Using: $\log Y = \log a + b \log X$. a and b = integers from Greenleaf <i>et al.</i> , (2007).	Establishes spatial scale at which bees can supply crop and wildflower pollination services (Kremen, 2005). Larger foraging range can rescue isolated flowering plant populations / allow for foraging in fragmented landscapes (De Palma <i>et al.</i> , 2015)
Tongue length	Quantitative, Mean (+/-SD) (mm)	Family specific trait (De Palmaet <i>et al.</i> , 2015). Length of prementum and glossa when tongue is fully extended (Nagamitsu <i>et al.</i> , 2007)	linked to resource partitioning and the pollen or nectar collection efficiency (Hanski, 1982; Treatt & Ackerly, 2013). Also, a morphological indicator of the type of resource use (Inouye, 1980). Trait varies specifically among bumblebees and longer tongued species are more specialised, selectively foraging on Fabaceae (Goulson <i>et al.</i> , 2005).
Individual level			
Behavioural traits	Time spent on flower (s)	A log of the time spent on the first flower in the quadrat in seconds	Differences in behaviour affects the effectiveness of bumblebees in pollinating wildflowers and crops (Wallace <i>et al.</i> , 2002). Due to recent human activity, ecological importance of including intraspecific variation into trait studies has increased (Roches <i>et al.</i> , 2018)

3.4 Statistical analysis

3.4.1 Biodiversity indices

To determine how foraging bumblebee biodiversity indices differ within a MFC, such as *Helianthus annuus*, regarding the control and the wildflower outcrop (the categorical explanatory main effect), a one-way ANOVA with Gaussian residual errors was carried out with abundance, richness and diversity as the continuous response variables. To calculate the comparisons between the three habitats (incrop, outcrop, control) for the significant variables, Tukey post hoc tests were completed. Normal assumptions for this test were met according to the Shapiro-Wilk and diagnostic plots. To reduce the risk of pseudoreplication and to account for density and plant composition variation between the fields, a split-plot ANOVA with Gaussian residual errors was carried out using Shannon diversity index as the continuous explanatory variable, and the field number (the fixed factor) and incrop or outcrop (repeated measured variable) as the categorical response variables.

3.4.2 Functional trait indices

Single trait analysis, utilising functional trait indices, Rao coefficient functional trait diversity (FD) and community weighted mean values (CWM), was undertaken (Mason *et al.*, 2005; Ricotta & Moretti, 2011; Pla, Casanoves & Di Rienzo, 2012). CWM has been defined as the trait average for each sample weighted by the species relative abundance, whereas FD shows how the trait diverges around this mean (Villéger, Mason & Mouillot, 2008; Ricotta & Moretti, 2011). Using Lepš *et al.*, (2006), FD and CWM values for each sample were calculated. The same splitplot ANOVA used for diversity was undertaken with CWM and FD of the selected community traits as the continuous explanatory variables.

3.4.3 Community composition and individual variation

To show whether the foraging bumblebee community composition was different within the sunflowers and to summarise the variation in the relative frequencies of the species a unimodal DECORANA ordination plot, TWINSpan and chi-squared tests were performed using the community analysis package CAP 4.0. All other statistical analyses were conducted with R version 3.1.1. (R Core Team, 2014). *Bombus spp.* within each community were individually examined on paired differences between the habitats, incrop and outcrop. Normality was tested using the Shapiro-Wilk test. As the majority of data was found to be non-normally distributed a Wilcoxon signed-rank test was used (Sokal & Rohlf, 2000).

To test the individual variation, the same split-plot ANOVA was executed with the log of the flower visitation length (sec) as the continuous explanatory variable. Interspecies variation within the flower visitation length analysis was controlled by adding species as another main effect to the split-plot ANOVA.

4. Results

Across the 40 transects including the control and the two sampling methods, 583 *Bombus* individuals, belonging to eight species were recorded. A total of 351 individuals were observed over 1,200 minutes, while 232 individuals were captured during the transect walks. The most abundant species were the species *B. terrestris* (26.8 %), *B. lapidarius* (27.4 %), and *B. pascuorum* (34.5 % of the walked transects) or the subgenus *Thoracobombus spp.* (41.9% of the observations), totalling 93.1 % of all individuals recorded (Appendix 4). These species along with *B. muscorum* (3.0% of the walked transects) were the only species to be found in the incrop and outcrop of the sunflower fields and the control.

4.1 Taxonomic and functional trait diversity

The bumblebee abundance, richness and diversity significantly varied across the incrop, outcrop and control (Figure 3), with the highest mean bumblebee abundance ($\bar{x} = 7.276 \pm 0.752$), richness ($\bar{x} = 2.897 \pm 0.224$) and diversity ($\bar{x} = 0.780 \pm 0.0683$) occurring within the sunflower crop. The lowest occurred in the control (abundance, $\bar{x} = 2.833 \pm 0.543$, richness, $\bar{x} = 1.5 \pm 0.224$, diversity, $\bar{x} = 0.303 \pm 0.137$). The sunflower incrop was significantly greater in all biodiversity indices than the outcrop and control (Table 3). When accounting for inter-field differences and excluding the control, the split-plot ANOVA still found the bumblebee diversity to still be significantly greater in the incrop than the outcrop ($F_{(1,4)} = 9.464, p = 0.022$), and the differences between each field to not impact the bumblebee diversity (Figure 4).

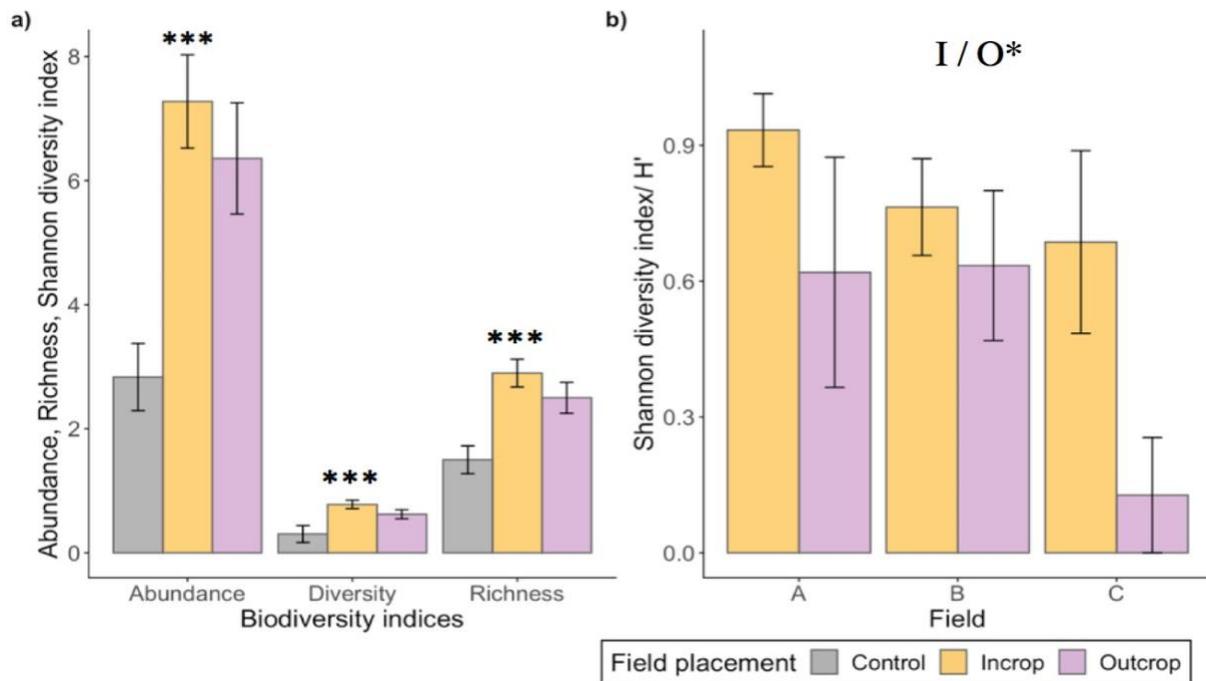


Figure 3: Mean biodiversity indices (\pm SE) across the areas studied. a) Abundance, diversity, richness within the incrop and outcrop of all the sunflower fields and the control (Total N = 40). b) Shannon diversity index within each incrop and outcrop of each field (Total N = 34). (A, B, C). I = incrop, O = outcrop (Wickham, 2009). Significance shown with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ utilising a) a one-way ANOVA, b) a split-plot ANOVA.

Table 3: One-way ANOVA results for bumblebee biodiversity indices across the three habitats, incrop, outcrop and control. Tukey HSD values shown for the significant ANOVA results. O = outcrop, I = incrop, C = control.

Biodiversity indices	ANOVA results		TukeyHSD <i>p</i> -values		
	F _(1,4) statistic	p-value	O / I	O / C	I / C
Abundance	21.830	< 0.001	< 0.001	0.926	< 0.001
Richness	13.510	< 0.001	< 0.001	0.781	< 0.001
Diversity	7.045	< 0.001	0.008	0.548	0.014

The incrop or outcrop variable and the inter-field variation did not significantly impact any of the functional trait indices (FD, CWM), apart from sex FD which was significantly greater within the sunflowers than the wildflower outcrop ($F_{(1,4)} = 11.937$, $p = 0.014$; Figure 4). Overall neither the incrop or outcrop variable or field significantly affected the sex CWM, though Figure 5 shows a higher proportion of female foraging within the wildflower outcrop in fields A and B.

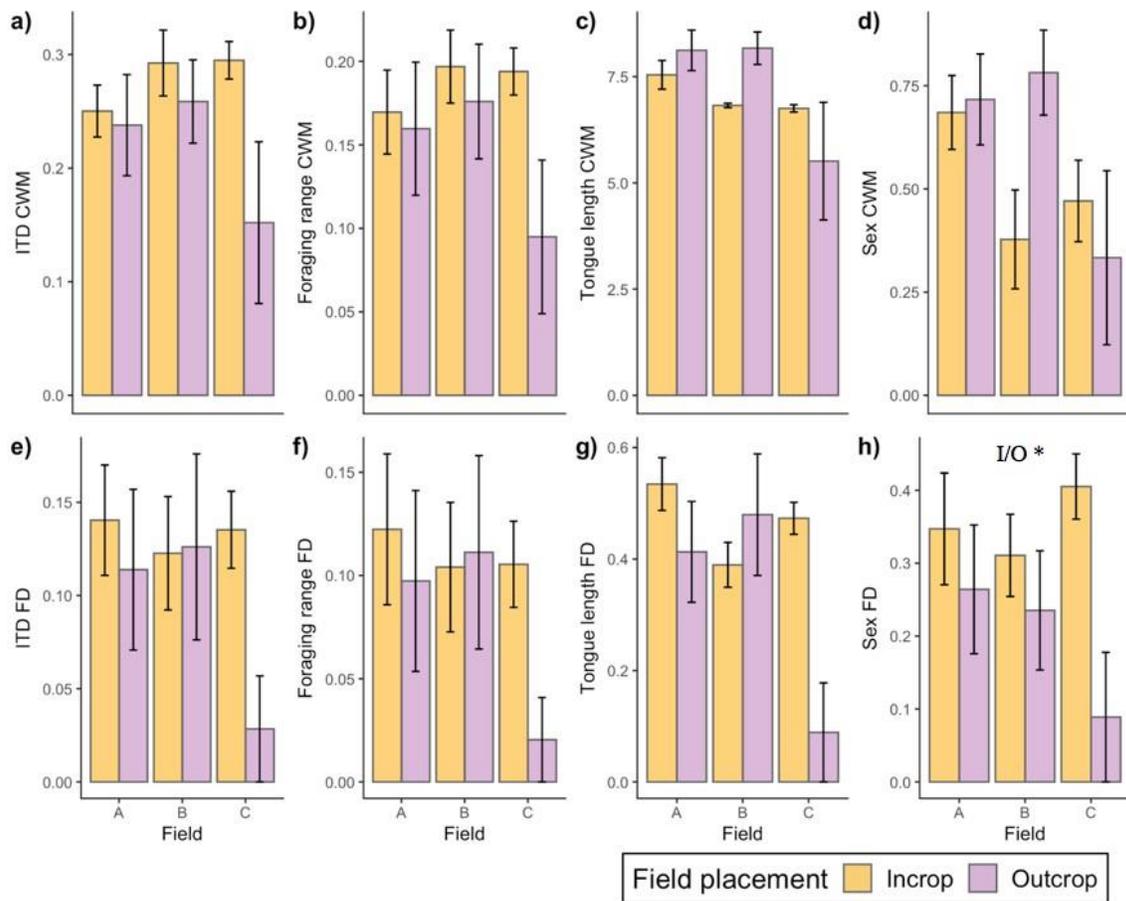


Figure 4: Functional trait indices (mean CWM and FD (\pm SE)) of bumblebee communities across the incrop and outcrop of three different sunflower fields (total N = 34). a) Intertegular distance (ITD) CWM (coded, 0 - minimum value, 1 - maximum value), b) foraging range CWM (coded, 01), c) Tongue length CWM (not coded and including \pm SD, (mm)), d) sex (0: male, 1: female), e) ITD FD, f) foraging range FD, g) tongue length FD, h) sex FD. I = Incrop, O = Outcrop. Significance shown with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ utilising a split-plot ANOVA.

4.2 Community composition

Associations between bumblebee species and the transect walk samples are shown in Figure 5, with the distance between samples indicating community composition differences and distances between samples and species demonstrating how specialist the feeding preference is towards a select habitat. Similarities in community composition are shown across the incrop, outcrop and control with *B. jonellus* only occurring within the incrop and *B. lapidarius* rarely within the outcrop (Figure 5A). However, when separating the field C outcrop from these communities, incrop samples cluster around *B. jonellus*, *B. lapidarius* and *B. terrestris*, and directly contrast with the outcrop samples (Figure 5B). When separating all of the fields, the incrop of field A clusters closer to the Field A and B outcrop than the other incrops and around *B. pascuorum* (Figure 5C).

B. terrestris was significantly positively associated with *B. lapidarius* ($\chi^2 = 11.193$, $p < 0.05$) but negatively associated with *B. pascuorum* ($\chi^2 = -6.508$, $p < 0.05$). This is supported by Figure 6 which shows three clear divisions based on the associations between the different species. Two distinct clades occur from the first division, separating species predominantly found foraging in the incrop (the upper clade) and the outcrop habitats (the lower clade). Two further divisions occur within the upper clade causing the species *B. terrestris* and *B. lapidarius* along with *B. jonellus* to be closer related to the upper clade and hence furthest in proximity from the species within the lower clade (Figure 6).

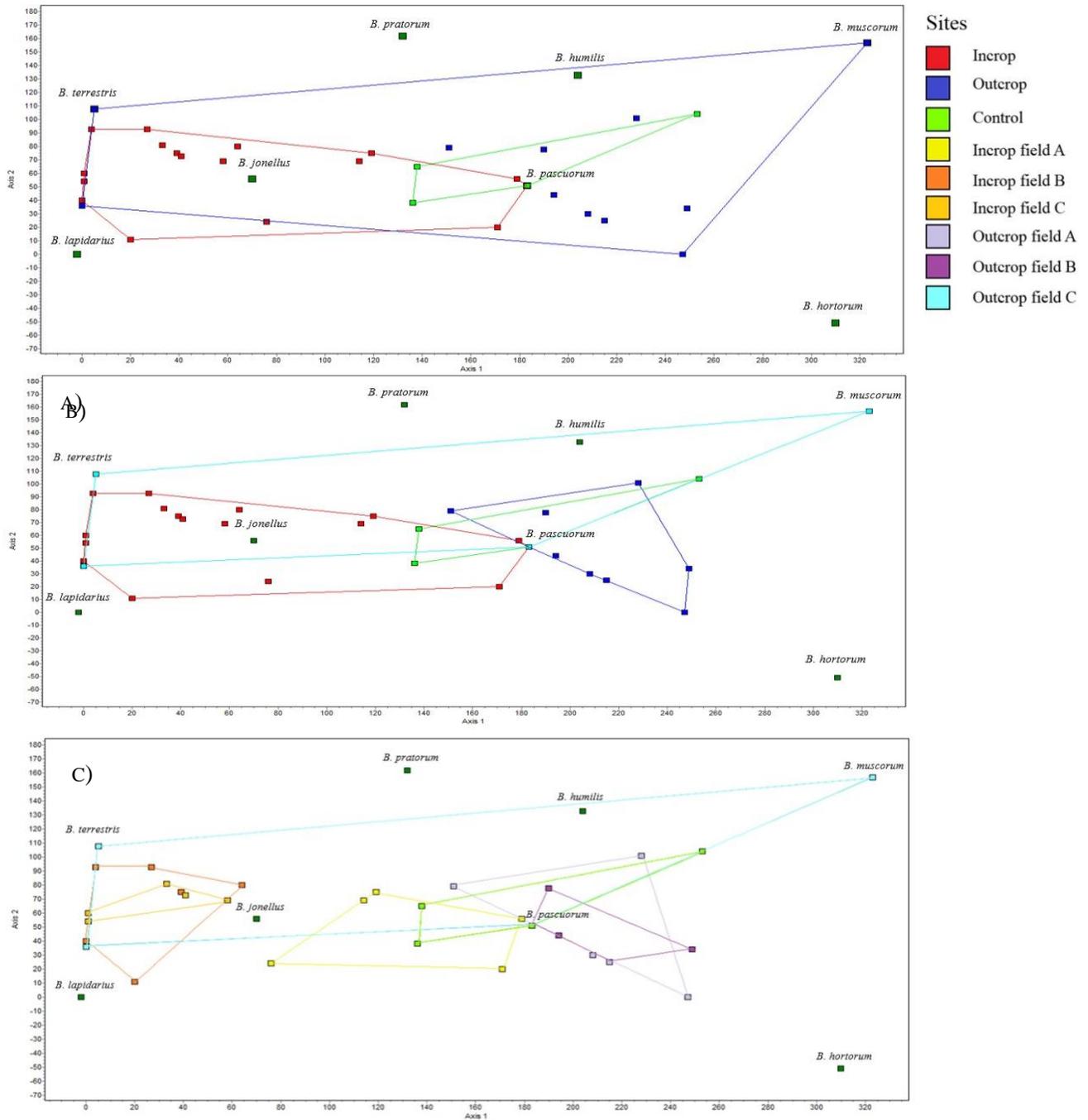


Figure 5: DECORANA ordination plot of the 40 bumblebee samples taken using the transect walk method at the Vile, Rhossili and Oxwich, South Wales, using CAP 4.0 software. A) Grouped incrop, outcrop and control, B) the incrop, outcrop (minus field C), control, and field C outcrop, C) the control, all fields, outcrops and incrops.

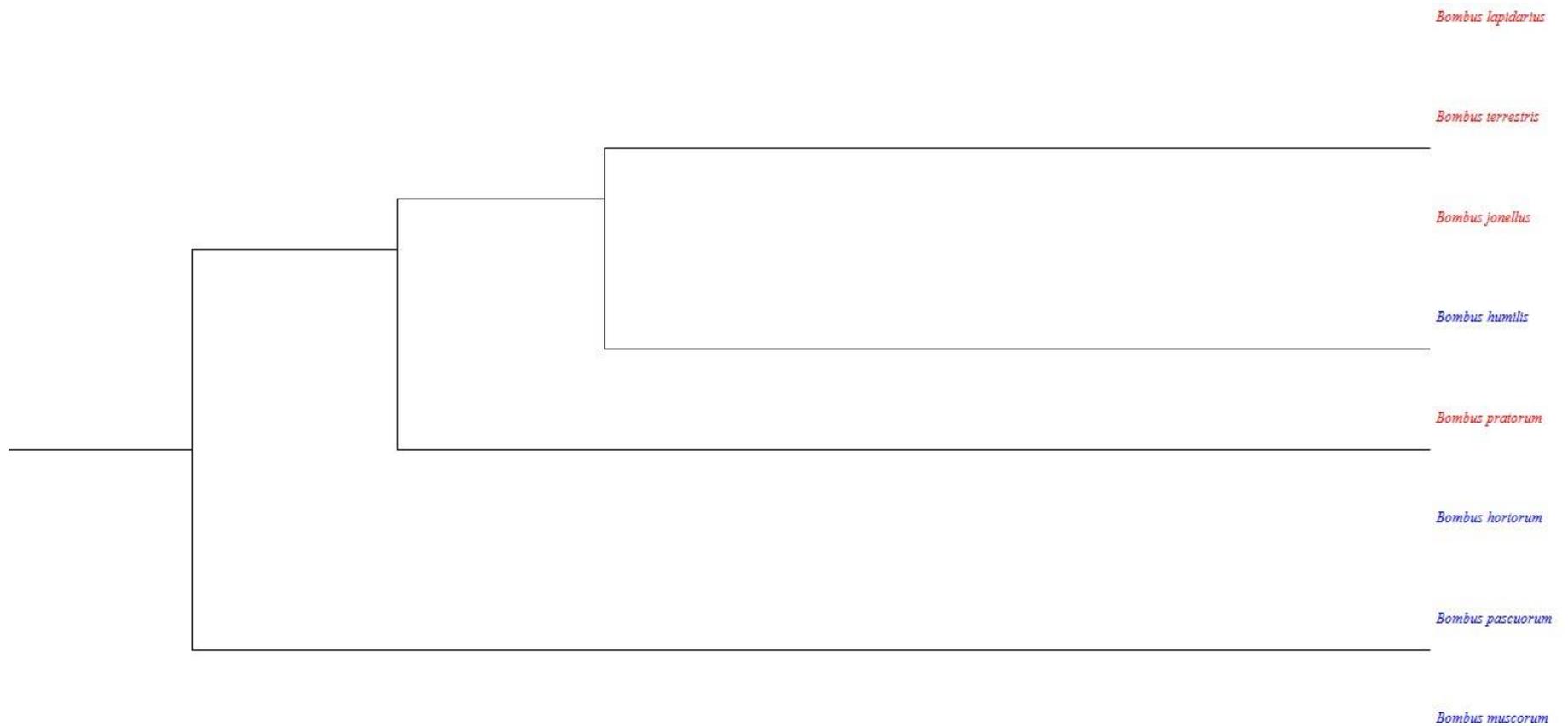


Figure 6: A three division hierarchical relationship between the eight species collected during the transect walk method and their relative proximity shown in a TWINSpan dendrogram plot using CAP 4.0 software. The colour does not imply upper or lower association or classification.

Across both sampling methods, *B. terrestris* and *B. lapidarius* were significantly higher in abundance in the sunflowers than in the wildflower outcrop (Figure 7). No species was significantly higher in the wildflower outcrop than the sunflower incrop, though *B. pascuorum* was found equally between the two habitats (Figure 7).

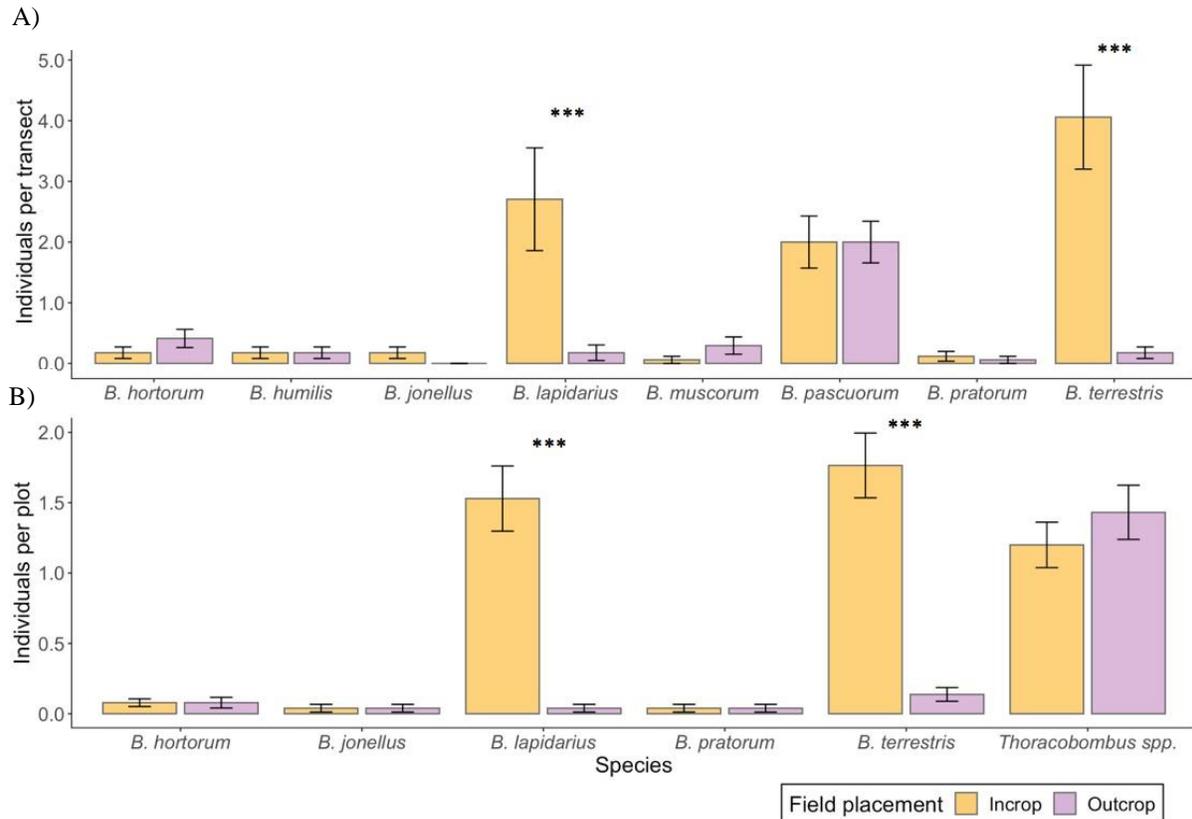


Figure 7: Mean number of *Bombus* spp. or subgenus (\pm SD) recorded at the Vile, Rhossili in relation to the crop and wildflower outcrop. A) Foraging species recorded during 15m transect walks (N = 34), B) foraging species or subgenus observed during ten minutes in 1 m x 1 m plots (N = 102). Significance shown using Wilcoxon rank-sum tests: * p < 0.05, ** p < 0.01, *** p < 0.001.

4.3 individual level behaviour

The greatest mean visitation length per flower head occurred within the sunflower crop ($\log \bar{x} = 2.35 \pm 1.30$) and the lowest occurred in the control ($\log \bar{x} = 0.808 \pm 0.502$). When solely observing the sunflower fields, the split-plot ANOVA found the mean visitation length to be significantly greater within the sunflower field in comparison to the wildflower outcrop and there was no difference between the three fields ($F_{(1,6)} = 11.378, p = 0.015$; Figure 8). However, when the species variation was controlled for within this model the difference in visitation length between the incrop and outcrop (Table 4) was not significant.

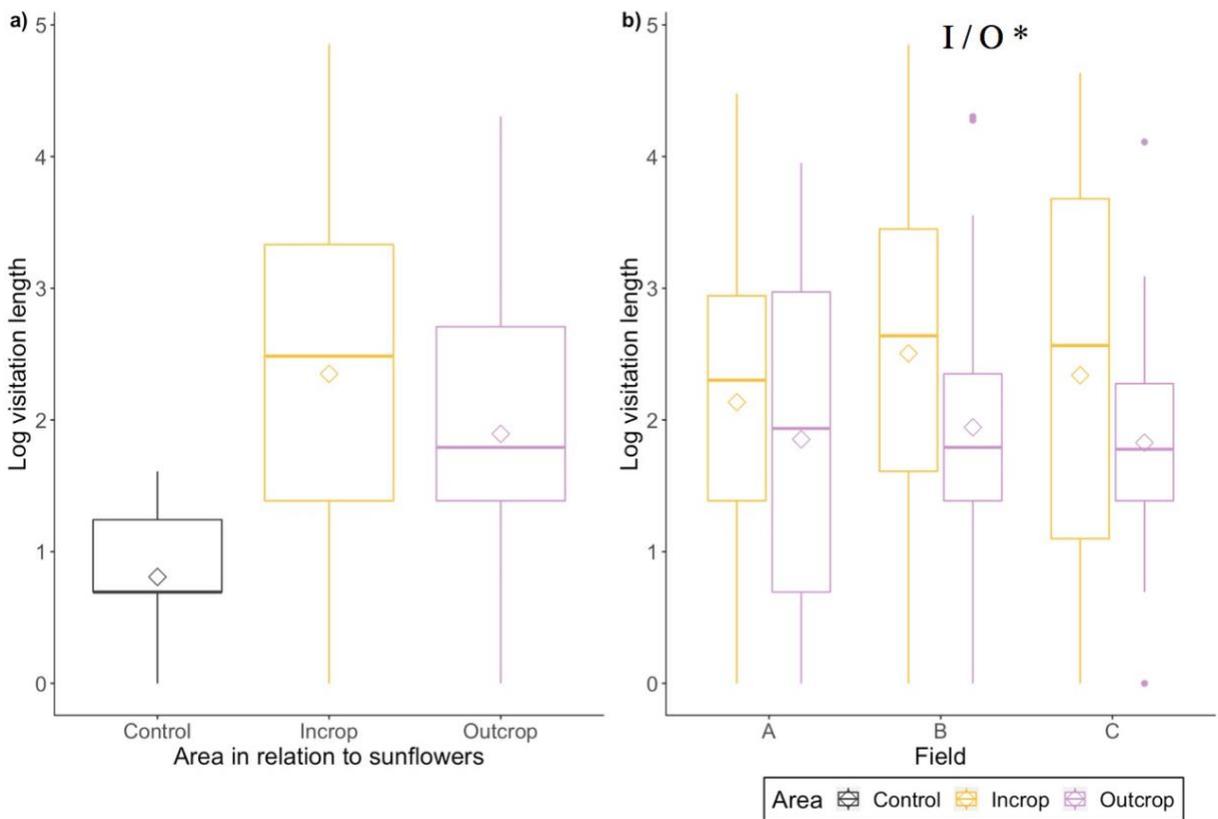


Figure 8: Log flower visitation length across the different areas studied (total $N = 232$). Interquartile range, median and mean (diamond) shown in boxes with whiskers indicating upper and lower boundaries. a) Incrop, outcrop of all sunflower fields and control, b) incrop and outcrop of each field (A, B, C). I = incrop, O = outcrop. Significance shown with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ utilising a split-plot ANOVA.

Table 4: Split-plot ANOVA results for bumblebee visitation length to flowers when species were and were not controlled for across the three fields and the incrop or outcrop as the repeated measure variable.

Visitation length	Incrop/outcrop			Field		
	F- statistic	DF	<i>p</i> -value	F-statistic	DF	<i>p</i> -value
Species not controlled for	11.378	1,6	0.015	3.798	2,6	0.086
Species controlled for	18.668	1,1	0.145	3.921	2,1	0.336

5. Discussion

5.1 Bumblebee diversity and community composition

The sunflower crop contained a higher abundance, species richness and diversity of foraging bumblebees than the wildflower outcrop and control, supporting this study's first hypothesis. This result parallels the findings by Todd, Gardiner & Lindquist (2016) and implies that the sunflowers at Rhossili benefit wild bumblebee communities, possibly due to the additional foraging resources sunflowers provide within the agroecosystem (Minckley *et al.* 1994). Further supporting Todd, Gardiner & Lindquist's (2016) findings, increased incrop diversity also occurred when accounting for inter-field variation in sunflower density and outcrop plant composition. This suggests that, independent of these factors sunflower integration into agroecosystems increases diversity within the crop.

Consequently, the rise in bumblebee abundance and diversity in relation to sunflower presence could positively impact the pollination of crops and surrounding wild plants (Westphal *et al.*, 2003; Hanley *et al.*, 2011). However, this suggestion is highly debated as some studies suggest that MFCs negatively promote increased competition between wild plant species through pollinator dilution (Holzschuh *et al.*, 2011; Holzschuh *et al.*, 2016), while others indicate that MFCs cause pollinator spill-overs into bordering semi-natural habitats (Hanley *et al.*, 2011; Rieginger *et al.*, 2014; Stanley & Stout, 2014). Unfortunately, potentially due to the small sample size in comparison to previous studies (Holzschuh *et al.*, 2016; Hanley *et al.*, 2011) and differences

in flower composition between the control and the outcrop (Appendix 2), the lack of significance between the control and outcrop habitats within this local study provides evidence for neither suggestion.

Overall, the community composition of the incrop and outcrop habitats did not drastically vary when not considering inter-field differences. However, this finding is skewed by the field C outcrop as when removed, the incrop and remaining outcrop habitats do contrast in community composition (Figure 5b). This finding provides evidence for Todd, Gardinerr & Lindquist (2016) suggestion that the combination of sunflower and bordering wildflower habitats is a more effective bumblebee conservation strategy than a sunflower monoculture as it supports a broader community of bumblebees within the agroecosystem. This result, expanding upon Todd, Gardinerr & Lindquist (2016), also highlights the importance of recognising the floral composition of the wildflower habitats within management strategies, as the *C. arvense* outcrop of Field C had a lower diversity and abundance but broader composition than the other *T. pratense* outcrops. Species rareness within the community composition also needs to be recognised, as locally rare species can bias results through distribution underestimation caused by low abundance within samples (Hughes, Daily & Ehrlick 2000). Hence, to compare compositional variations between communities accurately, differences in common species were primarily focused upon within this study.

Common species, such as *B. lapidarius*, *B. pascuorum* and *B. terrestris* are known to readily incorporate MFCs into their diets because of their generalist dietary preferences and large foraging ranges (Goulson *et al.*, 2002; Goulson *et al.*, 2006; Somme *et al.*, 2015). When viewing the species structure within the incrop and outcrop bumblebee communities, *B. terrestris* and *B. lapidarius* were found abundantly together and more within the sunflower incrop than the wildflower outcrop. This preference for the sunflower crop over the wildflower outcrop could be due to *B. lapidarius*' gravitation towards mass flowering plants, such as the Asteraceae family (Goulson & Darvill, 2004; Prys-Jones & Corbet, 2011), and *B. terrestris*' preference for horizontally facing flowers that allow for a considerable landing platform, as found in sunflowers (Prys-Jones & Corbet, 2011). *Bombus lapidarius* and *B. terrestris*' coexistence within the sunflower incrop could have been supported by the large quantities of easily accessible sunflower pollen and nectar (Minckley *et al.*, 1994). Following the niche partitioning concept, these species

should not be associated as they have similar functional traits (short tongue lengths, large body sizes, large foraging range) (Goulson & Darvill, 2004; Benton, 2006; Goulson *et al.*, 2008). However, locally high abundances of floral resources and spatio-temporal heterogeneity in the foraging resources over the season allow for these considerable overlaps in resource utilisation by *Bombus spp.* (Ranta & Vepsäläinen, 1981).

Though sunflower pollen is abundant and accessible, it is low in protein (Nicolson & Human, 2013). *Bombus pascuorum* actively forages for higher protein pollen than *B. terrestris* (Leonhardt & Blüthgen, 2012; Somme *et al.*, 2015). Thus, the need for a wider breadth of pollen quality would explain why *B. pascuorum*, unlike *B. terrestris* and *B. lapidarius*, was highly abundant in both habitats as the low-quality sunflower pollen alone could not fulfil *B. pascuorum*'s dietary preferences. The density of the sunflowers and hence the prominence of the wildflower undercrop could also explain this result as the Field A incrop was similar in bumblebee community composition to the Field A and B outcrop (Figure 5c). Regardless of either reasoning, these findings highlight the importance of an outcrop or a sufficiently dense wildflower under crop in encouraging *B. pascuorum* populations.

5.2 Ecosystem services through functional traits

Contrary to the taxonomic diversity indices which supported the first hypothesis, the functional diversity indices did not significantly differ between the incrop and outcrop and across the three fields, apart from the sex FD (Figure 4). This result, whilst also implying that there is considerable overlap in the services provided across the *Bombus* genus (Chao, Chiu & Jost, 2014), supports the idea that foraging bumblebee communities within the sunflower crop are more diverse, but concerning the ecosystem services they provide, functionally similar to the wildflower outcrop (Forrest *et al.*, 2015). These findings parallel those of Rader *et al.*, (2014) and Forrest *et al.*, (2015), who found a greater species richness of pollinators within organic agro-management schemes, but little variation in pollinator functional trait diversity.

Though previous studies support this overall result, the insignificant variation in specifically tongue length FD was unexpected as resource partitioning due to tongue length variation is known to be a primary factor in determining bumblebee diversity (Inouye, 1978; Hanski, 1982; Goulson *et al.*, 2005; Goulson, 2008). Additionally, *Bombus spp.* forage on

sunflowers irrespective of tongue length differences, attracting a large range of short and longtongued bumblebees (Hurd *et al.*, 1980; Prys-Jones & Corbet, 2011). Varying floristic diversity within the incrop and outcrop could be an explanation for these findings (Appendix 2), as it was not taken into consideration within the analysis due to the small sample size. Another possible explanation is limitations regarding the functional metrics. Functional diversity indices, such as CWM and FD, are highly sensitive to under-sampling and rare species with low abundances (Plas *et al.*, 2017). Though methods were taken to reduce this limitation, such as using functional trait diversity rather than compound indices of functional diversity, this study's low sample size, especially in field C outcrop, in comparison to other insect-based studies could have reduced the results precision (Ricotta & Moretti, 2011; Forrest *et al.*, 2015; Martins *et al.*, 2015; Plas *et al.*, 2017). If repeated to combat these possible imprecisions, multiple subsamples within longer transects should be utilised.

Irrespective of these limitations, sex FD was significantly greater within the sunflower incrop in comparison to the wildflower outcrop. This result, along with the sex CWM findings (Figure 4d), implies that the sunflower habitats across three fields had more even sex ratios. As a late-season crop (Riedinger *et al.*, 2014), the sunflower's flowering period aligns with male bumblebees' late summer courtship, causing an increased male-biased sex ratio across all sites to be expected (Goulson, 2010). However, rich flower sources attract queen bumblebees and cause males to strategically occupy the same resources (Jennersten, Morse & O'Neil 1991). Hence, male pre-mating behaviour could explain the more even sex ratio within the sunflowers (Goulson, 2010). Males and females differ in the mode and, depending on the plant's reproductive strategy, efficiency of pollen transport (Table 5; Ostevik, Manson & Thomson, 2010). A large male population can reduce the risk of colony extinctions (Goulson, 2010). Thus, by having a variation of this trait within the agroecosystem, the sunflower crop could positively support future bumblebee colonies and allow the provision of pollination services to a range of plants.

Females actively forage for the colony, whereas males forage for themselves while patrolling for queens, utilising flowers as resting points as well as foraging resources (Jennersten, Morse & O'neil, 1991). Therefore, differing sex ratios, along with the sunflowers larger floral area (Portlas *et al.*, 2018), could help to explain the greater visitation lengths found within the sunflower incrop. Although this result supports the second hypothesis which stated that the visitation length

would be greater in the sunflower incrop, this study found no evidence to suggest it was primarily due to intraspecific variation in behaviour. Intraspecific trait variation tends to be more significant in communities where an obvious trophic cascade is present, whereas interspecific trait variation is consistently found to be more important when direct interactions, such as bumblebees foraging on a resource, are concerned (Roches *et al.*, 2018). Hence, due to the lack of evidence for intraspecific variation within this study, future research into this topic should focus efforts on species and sex-specific functional traits rather than individual variation.

5.3 Sunflower management for bumblebee conservation

Sunflowers, as MFCs, only offer bursts of pollen and nectar at high quantities over short periods (Holzschuh *et al.*, 2016). This fact, along with this study's findings, highlights the importance of management in encouraging bumblebee populations and their regulating ecosystem services. To inspire agroecosystem resilience, semi-natural habitats are required alongside the crop to maintain bumblebee communities after the MFC season and as a precaution in case the crop fails to flower (Öckinger & Smith, 2007). Alongside this, *B. lapidarius* and *B. terrestris*' strong preference for the sunflower incrop, though wildflower habitats were available, needs to be considered in management schemes. Dependence on the sunflower's low protein pollen can impact bumblebee development by producing smaller colonies and larvae in the next generation (Nicolson & Human, 2013; Roger *et al.*, 2017). Possible suggestions to reduce the risk of nutrient deficiencies within these current and future populations include limiting the number of sunflower fields within an area and controlling all fields to a set time within the season.

As there was no difference in bumblebee diversity between the fields of varying sunflower density (Figure 3b), it can be argued that a more intensive sunflower incrop with a clear wildflower outcrop is more cost-effective. However, this study's focus was on bumblebees and did not take into consideration other insect pollinators such as solitary bees, hoverflies or butterflies. These important pollinator groups vary in ecological traits and therefore could respond to MFCs differently (Ekroos *et al.*, 2013; Kovács-Hostyánszki *et al.*, 2013). Hence, before this management strategy can be proposed, more research needs to be undertaken reviewing each pollinator groups diversity and functional diversity within the agroecosystem.

To build upon the study’s functional trait-based approach, this study’s results, alongside other agro-biodiversity management strategies findings, could be integrated into ecosystem service assessments through quantitative modelling (Laughlin, 2014; Figure 9). The procedure tests whether management strategies successfully attain the target distribution of functional traits within the agroecosystem and thus, achieve the intended ecosystem service rates (Laughlin, 2014; Wood *et al.*, 2015). Subsequently, future ecosystem service assessments could help to determine how this sunflower management strategy functionally compares to others and provide clearer quantitative targets to farmers (Wood *et al.*, 2015).

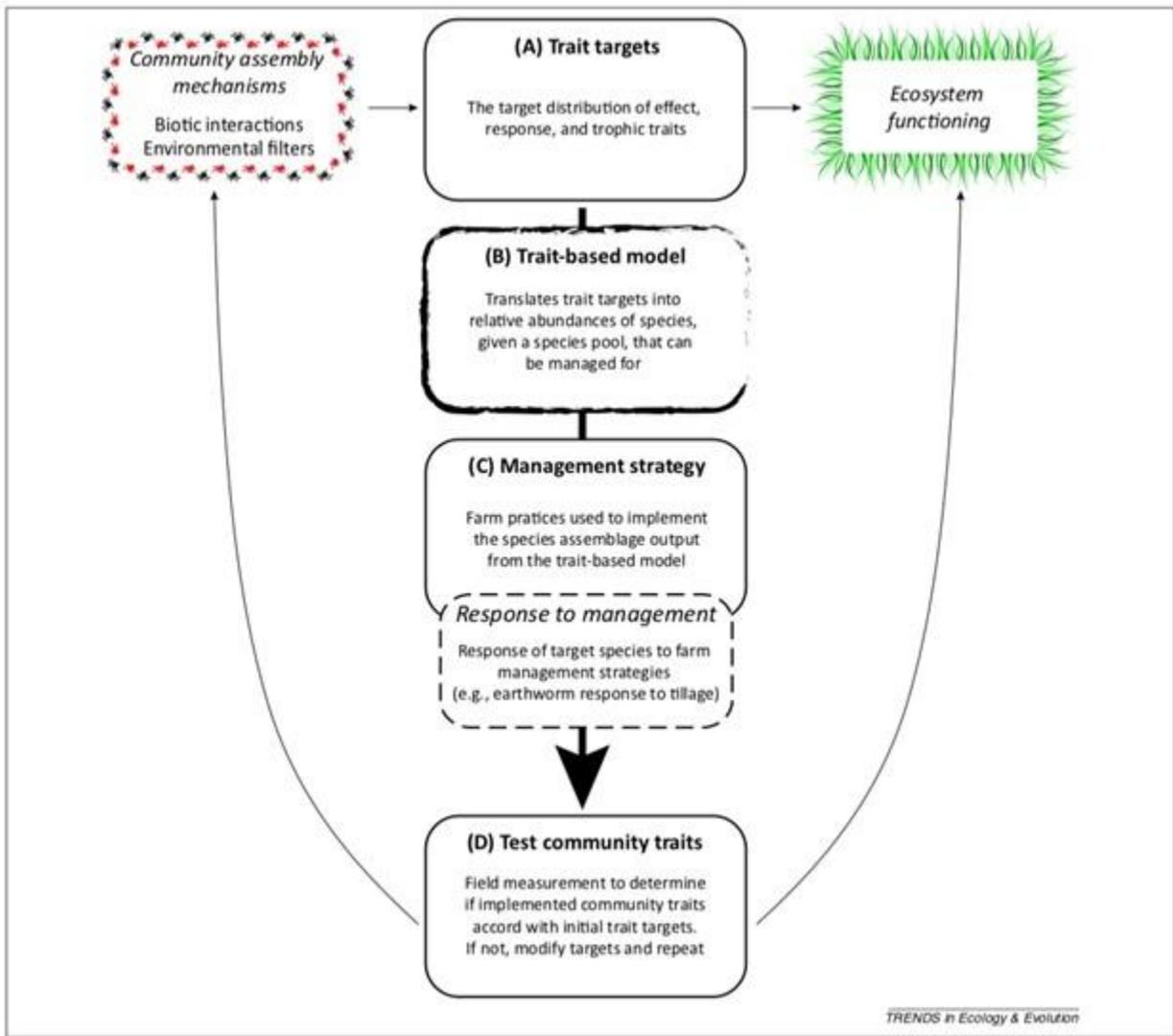


Figure 9: How functional trait targets can be transformed into agricultural management strategies using a modelled trait-based approach (Wood *et al.*, 2015).

This study's short duration, limited sunflower field repeats, limitations with the diversity matrices themselves and other limitations shown in Appendix 13, need to be taken into consideration alongside these findings and management suggestions. To monitor pollinator populations robustly and accurately, large, long-term pollinator studies (studies > 2 years and > 10 sites/ fields) are needed (Carvell *et al.*, 2017). Although the impacts of these limitations were minimised, more data would need to be collected across more sunflower fields and several years to verify these results with greater certainty.

6. Conclusion

Supporting Todd, Gardiner & Lindquist (2016), this study found a greater bumblebee diversity within the sunflower crop in comparison to the wildflower habitats and thus, demonstrated that sunflower crops can be utilised as a bumblebee conservation strategy. However, a wildflower outcrop, as an agro-ecological practice, is also required alongside the sunflower crop to encourage ecosystem resilience and future bumblebee populations (Öckinger & Smith, 2007). This allows for a broader bumblebee community composition across the agroecosystem and reduces the risk of nutritional deficiencies, caused by extensive sunflower foraging, within bumblebee populations (Roger *et al.*, 2017).

Concerning the pollination services which bumblebees provide, the communities within the incrop and outcrop were found to be functionally similar, with variations only occurring within the sex ratios. Although limitations regarding the length of the study need to be considered, this parallels previous agro-ecological management studies by highlighting that the management strategy appears to have little impact on the conservation of pollination services (Forrest *et al.*, 2015). To expand upon this approach, these findings along with other agro-biodiversity strategies should be incorporated into ecosystem service assessments (Wood *et al.*, 2015), thus allowing for functional comparisons between management strategies and clear targets for farmers.

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9. Appendix

9.1 Preliminary studies

The observer who undertook all of the data collection was trained to an intermediate level in bumblebee identification by the Bumblebee Conservation trust. Preliminary studies were conducted at Rhossili for a week prior to the 11th August 2019 to allow further bumblebee identification practise and to test the proposed method. Different capture methods proposed in the literature such as aerial netting, potting without aerial netting and sweep netting were tested. As aerial netting was the fastest and most accurate capture method with the lowest sampling error, it was chosen as the method of capture. However, though the sampling error for aerial netting was the lowest out of all of the method, during this preliminary study, errors still occurred. Hence, to not ignore this limitation, the proportion of bumblebees caught per transect was calculated during the whole data collection (Appendix 1).

Appendix 1: Sampling error detailing the number of bumblebees captured / the number observed within each transect.

Transect	Field A		Field B		Field C	
	Incrop	Outcrop	Incrop	Outcrop	Incrop	Outcrop
1	5/8	6/8	19/23	6/10	11/14	1/1
2	13/15	5/8	6/10	3/3	9/10	1/2
3	9/9	4/6	8/16	3/4	17/24	1/1
4	5/5	3/3	12/13	6/8	5/5	2/3
5	7/9	5/6	11/11	4/4	6/7	0/0
6	5/7	3/4	14/18	3/4	NA	NA
Mean proportion caught	0.83	0.77	0.77	0.8	0.85	0.79

9.2 Further method details

9.2.1 Sampling

The primary site, Rhossili, was a patchwork of different sized crop fields which ranged in size from 50 x 200 m to 100 x 300 m. Species of crops within different fields at the site included *Linum usitatissimum* (linseed), *Helianthus annuus* (sunflower) and *Triticum aestivum* (wheat) (estimated 0.1 km², 0.075 km², 0.16 km² in total area respectively). Though the majority of field studies on bees use a wind speed limit of 2.5 m/s, this environmental control was raised to 4.5 m/s because of the average coastal climate of the site. Before each transect the temperature and humidity was measured at a height of 2 m using a thermometer and the time of day recorded. Wind speed was monitored hourly during the sampling day following Hoehn *et al.* (2008). If rain did occur during a transect or observation, the sampling method in question was stopped and then repeated at least 20 minutes after the rain had ceased.

Within each transect two sampling methods were used, a transect walk and an observation survey. To allow for direct comparisons to occur between all traits at the two ecological levels, only using the transect walk would have been preferred (Woodcock *et al.*, 2013). However, within this study observer disturbance caused by moving through dense crops could have impacted the individual level behavioural responses and hence this part of the study was separated into a different method.

As the type of flowering species and their abundance was shown to affect the abundance and diversity of pollinators the flowering plant species and the number of flowers per species were recorded. This occurred in the same 3, 1 x 1 m quadrats as the individual level observations. The plant species richness and composition did vary across the three fields, along with the density of sunflowers (Appendix 2).

9.2.2 Functional traits and FD

Each species level functional trait was treated differently based on the data provided by Professor Stuart Roberts. Tongue length was treated as quantitative as standard deviation values were available. The ITD mean measurement and foraging range were treated as semi-quantitative (scaled between 0, the lowest value and 1, the highest) and sex was treated as binary (male, female: coded as 0 and 1 respectively).

Only single indices of functional diversity were attempted within this study. Past literature claims that assessing the distribution of values within single traits presents the most appropriate information for exploring the effects of the community composition on ecosystem functioning (Ricotta & Moretti, 2011). Hence following recommendations by Lepš *et al.*, (2006), a compound index of functional diversity was not attempted for this study.

Appendix 2: The flowering plants found at each incrop and outcrop within each field.

Field / Site	Incrop / outcrop	Flowering plants found in region
A	Incrop	<i>Glebionis segetum</i> , <i>Helianthus annuus</i> , <i>Polygonum aviculare</i> , <i>Taraxacum officinale</i> , <i>Trifolium pratense</i> , <i>Vicia cracca</i>
	Outcrop	<i>Calystegia sepium</i> , <i>Cirsium arvense</i> , <i>Convolvulus arvensis</i> , <i>Glebionis segetum</i> , <i>Polygonum aviculare</i> , <i>Taraxacum officinale</i> , <i>Trifolium pratense</i> , <i>Veronica persica</i>
B	Incrop	<i>Helianthus annuus</i> , <i>Trifolium pratense</i>
	Outcrop	<i>Linum usitatissimum</i> , <i>Solanum nigrum</i> , <i>Taraxacum officinale</i> , <i>Trifolium pratense</i> , <i>Trifolium repens</i> , <i>Veronica persica</i>
C	Incrop	<i>Cirsium arvense</i> , <i>Erigeron annuus</i> , <i>Helianthus annuus</i> , <i>Solanum nigrum</i> , <i>Taraxacum officinale</i> , <i>Trifolium pratense</i> , <i>Veronica persica</i>
	Outcrop	<i>Arctium minus</i> , <i>Cirsium arvense</i> , <i>Convolvulus arvensis</i> , <i>Erigeron annuus</i> , <i>Leontodon hispidus</i> , <i>Solanum nigrum</i> , <i>Taraxacum officinale</i> , <i>Trifolium pratense</i> , <i>Veronica persica</i>
Oxwich	Control	<i>Chamerion angustifolium</i> , <i>Lathyrus hirsutus</i> , <i>Oenothera glazioviana</i> , <i>Rubus fruticosus</i> , <i>Senecio erucifolius</i> , <i>Spiranthes spiralis</i> , <i>Taraxacum officinale</i>

9.3 Data sets

Appendix 3: Abundance of each species during the transect walks across all sites where data collection occurred in Rhossili and Oxwich from 11th August to 27th August 2019.

Species	Field A		Field B		Field C		Oxwich
	Incrop	Outcrop	Incrop	Outcrop	Incrop	Outcrop	Control
<i>B. lapidarius</i>	7	0	23	1	16	2	1
<i>B. hortorum</i>	2	3	1	4	0	0	0
<i>B. pascuorum</i>	21	17	6	16	7	1	12
<i>B. terrestris</i>	4	1	37	0	28	2	1
<i>B. humilis</i>	3	1	0	2	0	0	0
<i>B. pratorum</i>	1	1	1	0	0	0	0
<i>B. jonellus</i>	1	0	1	0	1	0	0
<i>B. muscorum</i>	0	2	1	2	0	1	1

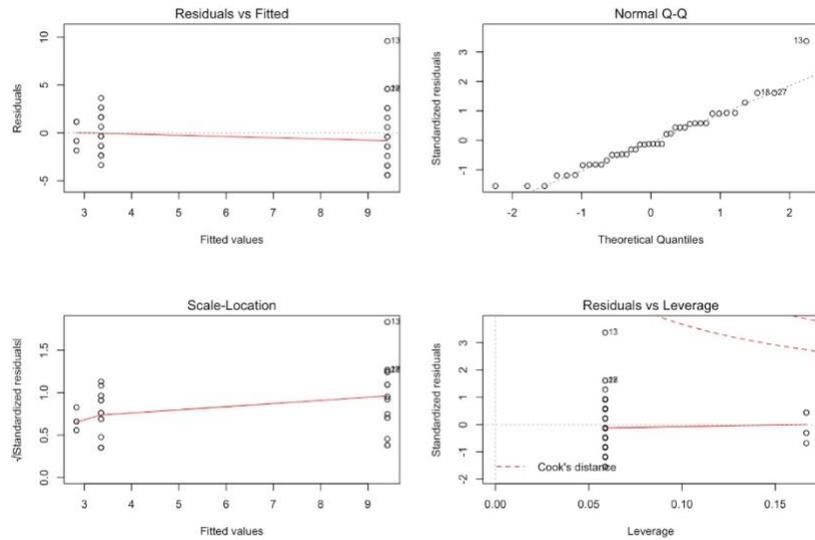
Appendix 4: Abundance of each species during the observations across all fields/sites where data collection occurred in Rhossili and Oxwich from 11th August to 27th August 2019.

Field/ Site	Area in relation to crop	<i>B.</i> <i>lapidarius</i>	<i>B.</i> <i>hortorum</i>	<i>B.</i> <i>terrestris</i>	<i>B.</i> <i>pratorum</i>	<i>B.</i> <i>jonellus</i>	<i>Thoracobombus</i>
A	Incrop	21	4	18	1	0	28
	Outcrop	0	2	4	0	2	18
B	Incrop	32	0	44	1	1	23
	Outcrop	0	2	0	0	0	40
C	Incrop	25	0	28	0	1	9
	Outcrop	2	0	3	2	0	15
Oxwich	Control	5	0	7	0	1	14

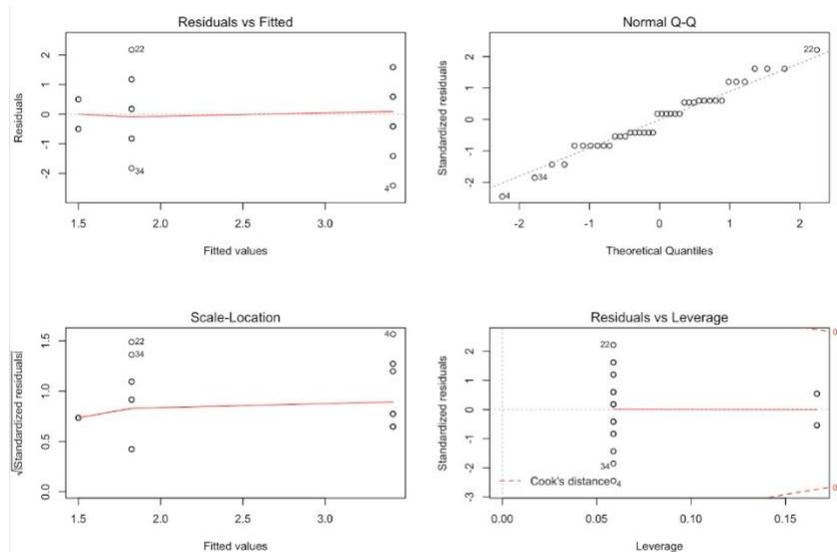
Appendix 5: Number of individuals of each species found at each location (incrop, outcrop of each field and control within Rhossili and Oxwich) from 11th August to 27th August 2019.

Field/ location	Incrop/ outcrop / control	Total abundance (Transect)	Total abundance (Observation)	Total Proportion (%)
A	Incrop	39	68	18.4
	Outcrop	26	26	8.9
B	Incrop	69	101	29.2
	Outcrop	25	50	12.9
C	Incrop	52	63	19.7
	Outcrop	6	15	3.6
Oxwich	Control	15	27	7.2

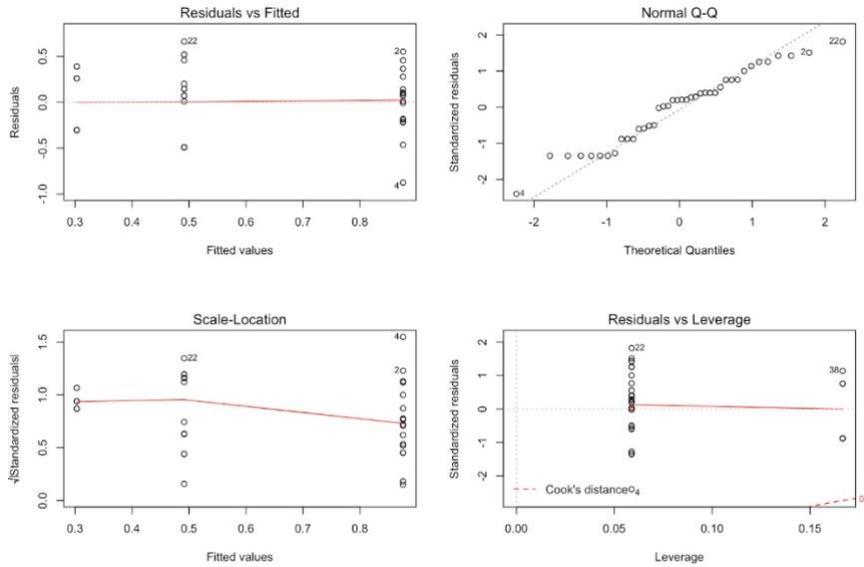
9.4 Shapiro-Wilk test results and diagnostic plots



Appendix 6: diagnostic plots for the one-way ANOVA results for the abundance of bumblebees between the incrop and outcrop. Normality also shown in the results of the Shapiro-Wilk test ($W = 0.94528$, p -value = 0.05223)



Appendix 7: diagnostic plots for the one-way ANOVA results for the bumblebee richness between the incrop and outcrop. Normality is also shown in the Shapiro-Wilk test results ($W = 0.97566$, p -value = 0.5323).



Appendix 8: diagnostic plots for the one-way ANOVA results for the bumblebee diversity between the incrop and outcrop. Normality is also shown in the results of the Shapiro-Wilk test ($W = 0.96144$, $p\text{-value} = 0.1875$).

Appendix 9: Shapiro-Wilk test results showing normality which using a t-test to test the difference in abundance of each species between in the incrop and outcrop. Normal distributed tests are highlighted.

Species	W - statistic	p - value
<i>B. jonellus</i>	0.47016	< 0.001
<i>B. lapidarius</i>	0.76042	< 0.001
<i>B. terrestris</i>	0.93859	0.302
<i>B. muscorum</i>	0.49008	< 0.001
<i>B. pascuorum</i>	0.93386	0.252
<i>B. pratorum</i>	0.5936	< 0.001
<i>B. hyptorum</i>	0.77439	< 0.001

9.5 Further Statistical tables

Appendix 10: Split-plot ANOVA results for bumblebee indices (diversity using Shannon diversity index, CWM and FD of the selected traits) across the three fields and incrop or outcrop.

Bumblebee indices	Incrop/outcrop		Field		Interaction	
	F _(1,4) statistic	p-value	F _(2,4) statistic	p-value	F _(2,4) statistic	p-value
Diversity	9.464	0.022	1.75	0.252	2.321	0.179
CWM ITD	2.18	0.19	0.588	0.584	0.98	0.429
CWM foraging range	1.524	0.263	0.542	0.608	0.701	0.533
CWM tongue length	0.217	0.657	2.244	0.187	1.233	0.356
CWM sex	1.909	0.216	2.242	0.187	3.772	0.12
FD ITD	1.258	0.305	0.786	0.515	0.78	0.518
FD foraging range	0.739	0.423	0.621	0.582	0.492	0.644
FD tongue length	2.969	0.136	2.501	0.197	3.475	0.133
FD sex	11.937	0.014	0.588	0.597	3.072	0.155

Appendix 11: Chi-squared results for species occurring across all sites including the control with significant associations between species in bold. Calculated using CAP 4.0 software.

Species	<i>B. lapidarius</i>	<i>B. hortorum</i>	<i>B. pascuorum</i>	<i>B. terrestris</i>	<i>B. humilis</i>	<i>B. pratorum</i>	<i>B. jonellus</i>	<i>B. muscorum</i>
<i>B. lapidarius</i>								
<i>B. hortorum</i>	-0.249							
<i>B. pascuorum</i>	-3.12	0.271						
<i>B. terrestris</i>	11.193	-1.59	-6.508					
<i>B. humilis</i>	-0.0574	-0.0148	0.868	0.423				
<i>B. pratorum</i>	0.0193	-0.0752	-0.0752	0.0193	0.0041			
<i>B. jonellus</i>	1.808	-0.0752	-0.0752	0.0193	-0.0041	0.369		
<i>B. muscorum</i>	-0.0574	0.0148	-0.0148	-1.277	-0.271	2.991	0.0041	

Appendix 12: Wilcoxon rank-sum results for the difference in abundances of each bumblebee species (and one subgenus) between the incrop and outcrop of the sunflower fields at the Vile, Rhossili for each sampling method.

Species/ subspecies	Transect		Observation	
	V-statistic	<i>p</i> -value	V-statistic	<i>p</i> -value
<i>B. jonellus</i>	6	0.149	5	1
<i>B. lapidarius</i>	120	< 0.001	589.5	< 0.001
<i>B. terrestris</i>	118	0.001	733	< 0.001
<i>B. pratorum</i>	4	0.773	5	1
<i>B. hortorum</i>	7	0.2402	10.5	1
<i>Thoracobombus spp.</i>	N/A	N/A	322	0.332
<i>B. pascuorum</i>	145	1	N/A	N/A
<i>B. muscorum</i>	0	0.174	N/A	N/A
<i>B. humilis</i>	10.5	1	N/A	N/A

9.6 Study Limitations

Appendix 13: Description of this study's limitations, what was done to reduce them and what would be done if this study was repeated with limitless resources.

Limitation	Description	Done to reduce impact	Perfect experiment (nothing limited)
Sample size	Many variables for a low sample size	Controlled for as many variables as possible (time of day, wind, temperature)	Longer paired transects, split up into 3 sections. Repeat transect 3 times over collection period.
Control	Less transects, differences in site, flower composition and density.	Chose coastal habitat with similar environmental conditions	Two wildflower fields at Rhossili, one with primarily red clover and the other thistle. Paired transects taken around outside of field.
Pseudo replication	Lack of field repeats, skews results.	Utilised split-plot experimental design.	Nine fields, three repeats for the three field types
One observer	Not a direct comparison between incrop and outcrop.	Paired transect completed together with the incrop occurring either straight after or before outcrop.	Two observers completing incrop/outcrop transect together at the same time on all fields.
Tourist caused destruction	Trampling of flowers: incrop and outcrop. Increased over flowering period.	Selectively chose transects not disturbed by visitors, removed transects that were.	No human visitors.
Time of season	Sunflowers only flowering in August - impact composition of bumblebees.	Could not reduce limitation, instead a factor to consider.	Repeat over years and at different times of season (April, June, July, etc.)
Worker <i>B. terrestris</i> and <i>B. lucorum</i> differentiation	No differentiation in the field. Species have similar functional traits, but misidentification could impact diversity results.	Labelled as <i>B. lucorum terrestris</i> in the field. As a known rare species on site, recorded all as <i>B. terrestris</i> .	Collect all specimens and genetically ID each individual.
Time and area	Only occurred over one sunflower season and in Rhossili.	Highlighted that this occurred in Rhossili and not everywhere	Same experiment in different sites around country (not always coastal)

