



# Sustaining multiple ecosystem functions in agricultural landscapes: Effect of summer cover crops on weed control, soil quality and support to pollinators

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## ABSTRACT

Cover crops can perform multiple ecosystem functions, including weed control, soil quality enhancement and support to pollinators. While the contribution of individual cover crop species or mixtures to each of these functions has been extensively investigated, experiments testing all these ecosystem functions simultaneously to select the best species or mixture overall are rare. In this study, we evaluated the performance of six summer cover crop species in terms of biomass production, weed suppression, soil fertility enhancement potential and support to wild and managed pollinators. Tested species included buckwheat (*Fagopyrum esculentum* Moench.), white mustard (*Sinapis alba* L.), berseem clover (*Trifolium alexandrinum* L.), blue tansy (*Phacelia tanacetifolia* Benth.), fenugreek (*Trigonella foenum-graecum* L.) and common vetch (*Vicia sativa* L.). Field work was carried out in four fields in Northern Italy during the summer months of 2020 and 2021. Buckwheat was identified as the overall best-performing species, by virtue of its high biomass production, ability to control weed growth, and abundant and long-lasting flowering that could support honeybees and hoverflies during a low-resource period without promoting competition. Buckwheat was, however, less promising regarding soil enhancement potential and support to wild bees, highlighting the need to continue searching for complementary cover crop species to be used alongside it in a mixture. Our results could improve cover crop selection schemes, suggesting the necessity for a comprehensive approach aimed at enhancing multiple ecosystem functions in agroecosystems.

## 1. Introduction

Cover crops represent an increasingly important tool for enhancing productivity and ecosystem functions in sustainable agriculture (Daryanto et al., 2018). They are employed both in organic and conventional cropping systems (Wayman et al., 2017) and have a variety of functions depending on the species and the context, ranging from soil erosion mitigation (De Baets et al., 2011) to wildlife enhancement (Carpio et al., 2018). Many cover crop species are already well known for their usefulness in performing individual functions, with winter cover crops being particularly well studied and widely used for the improvement of water quality, soil nutrient content and soil physical features (Dabney

et al., 2001; Marcillo and Miguez, 2017; Sainju and Singh, 1997). The selection of the most convenient cover crop species or mixtures for a specific agroecological context would however require the evaluation of multiple functions simultaneously, a task that is rarely, if ever, carried out (Bryan et al., 2021; Candelaria-Morales et al., 2022; Ripoche et al., 2021).

In this respect, summer cover crops are gaining increasing interest as they can perform a variety of functions including weed control and soil organic matter enhancement (Blanco-Canqui et al., 2012; Isik et al., 2009; Weiler et al., 2018). Among summer cover crops, flowering species might prove particularly suitable for supporting multiple ecosystem functions simultaneously, as they not only provide soil cover and

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primary biomass, but they also represent a potential food source for the support of wild and managed pollinators. Summer is a critical period both for pollinators, due to food scarcity (Balfour et al., 2018; Timberlake et al., 2019), and for weed control, due to the rapid spread of invasive summer weeds (e.g. C4 plants) (Fried et al., 2019), making the identification of multifunctional cover crops addressing these issues especially important. In temperate regions, the use of flowering species as summer cover crops also involves some critical issues linked to the short inter-crop time (ca. three months), high temperatures and low precipitation regime (Tribouillois et al., 2016). For these reasons, field experiments aimed at identifying the most suitable summer-flowering cover crop species are urgently needed.

Weed suppression and soil fertility enhancement are among the most important functions performed by widespread cover crop species. Weeds are the most significant biotic factor limiting yields worldwide (Oerke, 2006) and herbicide-based solutions have several negative consequences including environmental pollution and induction and spreading of herbicide resistance in target weed populations (Annett et al., 2014; Délye et al., 2013). Thus, there is a great interest in finding sustainable weed control strategies that do not heavily rely on herbicide use. For instance, limiting weed seed production in the fallow period might greatly contribute to depleting weed seed banks and, hence, weed germination and growth as part of an integrated weed management strategy (Kumar et al., 2019). This could be achieved by using cover crops able to outcompete weeds (Cechin et al., 2022; Mirsky et al., 2010). The effectiveness of cover crops in competing with weeds for space and resources depends on some crucial crop traits. Allelopathic effects are particularly studied, although mostly on a limited set of well-known species (Kunz et al., 2016; Šćepanović et al., 2021; Sturm et al., 2018). Other factors including germination modality and timing, as well as growth rate, could also play an important role. For instance, early germinating cover crops that can rapidly occupy and/or shade soil before weed germination are expected to have an edge in weed suppression (Brust et al., 2014; Mirsky et al., 2011). Comparing such features between different cover crop species is thus essential for biological weed control, especially in the case of the often highly competitive summer weed species (Masin et al., 2006; Osipitan et al., 2019).

The improvement of soil fertility through cover crops is perhaps even more studied (Adetunji et al., 2020; Dabney et al., 2001; Fageria et al., 2005), with plant carbon-to-nitrogen ratio (C/N ratio) and C and N isotopic signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) being considered good predictors of the organic matter quality added to the soil (Boscutti et al., 2020; Creamer and Baldwin, 2000; Lowry and Brainard, 2016; Pellegrini et al., 2021), and plants such as legumes (Fabaceae) being especially important for enriching soil with nitrogen (Smith et al., 1987).  $\delta^{13}\text{C}$  is also a good indicator of water stress (Dercon et al., 2006), which is another key element in the competition against weeds (Patterson, 1995), especially during summer months. Therefore, isotopic analyses of both cover crops and weeds become very important to fully understand the competitive interaction between the two. Moreover, these measurements provide a direct comparison of the quantity and type of C and N apported to the soil by the standing biomass provided by cover crops and weeds.

Cover crops also represent potential food resources and shelter for wildlife, including ecosystem services providers such as pollinators. Insect pollinators, are facing a steep decline worldwide (van der Sluijs and Vaage, 2016; Zattara and Aizen, 2021) which represents a major problem for both natural habitats and agriculture, as pollinators are instrumental for the reproduction of the vast majority of terrestrial plants and are necessary or play a role in the yield of over 70% of the world crops (Ollerton et al., 2011; Vasiliev and Greenwood, 2020). The decline is due to a combination of factors including pesticide use, invasive species, pathogen spread and, most importantly, habitat and floral resources destruction (Ferreira et al., 2013; Vanbergen and Initiative, 2013). In this respect, flowering cover crops can represent an alternative food source for pollinators (Mallinger et al., 2019), encourage them to spill over to nearby cash crops and perform

pollination (Riedinger et al., 2014), and support them in absence of other flowers due to landscape degradation or seasonality – for instance during the summer period, when many insect populations increase (Candelaria-Morales et al., 2022) and flowers in agroecosystems become scarcer (Balfour et al., 2018). Ideally, pollinator management measures should support both managed honeybees (*Apis mellifera* L.) – often seen as the single most important insect species for crop pollination (Abrol, 2012) - and wild pollinators, which as a group are thought to give an equal or even larger contribution to pollination if compared with honeybees (Breeze et al., 2011; Garibaldi et al., 2013, 2014). Reaching this goal can sometimes be challenging, as managed honeybees can compete with wild pollinators and have a detrimental effect on their communities (Magrath et al., 2017; Ropars et al., 2019).

In this study, we set a field experiment in temperate agroecosystems of Northern Italy, which is among the most intensive agricultural areas in Europe (Rega et al., 2020), representing an archetypical European productive system in terms of environmental and agricultural conditions (Levers et al., 2018; Rendon et al., 2022). We investigated the potential of six summer flowering cover crop species as providers and supporters of multiple agronomical and ecosystem functions, including cover crop biomass production, weed control and soil fertility enhancement. On tested species that reached the flowering stage, we also monitored pollinator visits, relating their abundance to flower cover at the anthesis, pollinator diversity and honeybee density. The protocol proposed in this paper could be applied in different agroecological contexts to pinpoint the best cover crop species or mixtures performing a variety of ecosystem functions.

## 2. Materials and methods

### 2.1. Experimental design

The study took place in 4 fields located in the Udine Province, NE Italy (Table S1), investigated from June to September in both 2020 and 2021. The area is an agricultural lowland characterised by temperate climate, with a mean annual precipitation of ca. 1500 mm and a mean annual temperature of ca. 13.5 °C. During the summer months (June–September), when the experiment took place, the total precipitation for the area is ca. 540 mm and the mean temperature is ca. 21.5 °C. Climate data were obtained from the nearest official meteorological station of Fagagna (Udine, Italy), period 1990–2021, monthly values for the period and experimental year are fully reported in Table S2.

In 2020, in each of the tested fields we set up 18 adjacent 6 × 10 m experimental random plots to investigate 6 different cover crop species. Each cover crop species was sown on tilled soil in the last week of June in 3 randomly distributed plots out of the 18 in each field (3 repetitions per field), using a plot seeder. Tested cover crops were chosen mainly for their flowering intensity and duration potential, for pollinator support. The chosen cover crop species for 2020 were buckwheat (*Fagopyrum esculentum* Moench.), white mustard (*Sinapis alba* L.), berseem clover (*Trifolium alexandrinum* L.), blue tansy (*Phacelia tanacetifolia* Benth.), fenugreek (*Trigonella foenum-graecum* L.) and common vetch (*Vicia sativa* L.). Species details and seed density are reported in Table S3. A 4 m buffer strip of common vetch was sown at field margins, to homogeneously contain weed growth on the bare soils, ensuring the environmental homogeneity between the immediate surroundings of each tested field. As described in the results section, many cover crop species were not able to reach the flowering stage, showing a scarce attitude for pollination sustainment. This was probably due to species life cycle and its interaction with the severe competition from weeds, which ended up impairing their flowering. Consequently, based on the 2020 flowering performance, only buckwheat and white mustard were sown in 2021 for the pollinator monitoring experiment, with each species once again having 3 repetitions (6 × 10 m experimental random plots) per field. In both years, a metal pole was placed approximately at the centre of each plot, serving as a reference to place a 1 × 1 m plastic square delimiting

the area in which data would be gathered. As common vetch did not reach flowering in either year, the vetch buffer strip did not influence pollinator surveys on buckwheat and white mustard.

## 2.2. Plant cover, biomass and isotopic analysis

Data on plant cover, biomass production and isotopes were gathered both for cover crops and weeds, in order to better compare their competitive success.

Three photographic surveys of the vegetation in each plot were carried out in 2020, approximately every 3 weeks from July to September. Photographs were taken orthogonally to the surface of the 1 × 1 m area delimited by the plastic square placed using the metal pole as a reference. Photos were taken at a constant height of 200 cm above ground. Cover crop and weed cover was measured by photo digitalization, using the ImageJ® software (Schneider et al., 2012). For each photograph, plants were firstly separated from the soil through a colour threshold, then the area covered by each plant type was manually selected by polygons. Cover area of each plant type was automatically calculated by setting the photo scale using, as reference, the plastic frame length (100 cm). Soil cover during the first survey was considered as a proxy for germination time and rate of each cover crop, with fast and early germinating crops being expected to have a higher soil cover early after sowing.

In September 2020, at the end of the first-year experiment, all above-ground plant biomass was harvested from the 1 × 1 m areas delimited using the metal poles in each plot as reference. The plots (3 repetitions per crop species and field) were the same that were subjected to the photographic surveys because one of our aims was to link germination time and growth rate (i.e. plant cover) with the final biomass production. Cover crop and weed biomass was weighted separately on field for each plot. Dry weight was measured after oven-drying biomass at 70 °C for 72 h. The idea of harvesting biomass multiple times during the season to coincide with photographic surveys was abandoned, as it would have required to sample different 1 × 1 m areas each time in each plot. This would increase the spatial plot heterogeneity by creating gaps in the canopy, and moreover the occurring heterogeneity in weed and cover crop distribution and productivity would have cast doubt on the validity of such data (Maestrini and Basso, 2018; Martín et al., 2015).

The dry biomass of weeds and cover crops was then used to measure the C and N content and C and N isotopic signature ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively) of leaves. Each dry sample was homogenised using a grinding mill. Then, a subsample was ball-milled and used for the analysis. The C and N content and isotopic signature analysis was performed using a CHNS Elemental Analyser (Vario Microcube, © Elementar) coupled to a stable isotope ratio mass spectrometer (IRMS; Isoprime 100, © Elementar).

## 2.3. Pollinator surveys

Pollinator visit surveys were carried out in 2021 every 1–2 weeks from the end of July (the beginning of flowering) to the end of August (right before cover crop harvesting) – for a total of 4 sampling rounds across one month - in all plots that reached the flowering stage. Surveys always took place between 8:45 and 11:00 a.m. in windless, sunny weather conditions. In each plot, a plastic square was placed on the vegetation using the metal pole as a reference.

Visual surveys were initiated 60 s after the placing of the square and lasted for 5 min each. During this time, we recorded the number of floral visits per insect pollinator species occurring within the square. A visit was considered as any instance in which a pollinator landed on a flower and touched its reproductive parts – consequently, a single individual could perform multiple visits by landing on multiple flowers. In case of pollinator morphospecies which could not be reliably identified on field, one or more individuals were caught and identified later by experts based on morphological characters. Flower cover was roughly

homogeneous among all surveyed buckwheat plots, as it was among all surveyed white mustard plots.

It must be remarked that many flowering weed species can provide important food resources for pollinators (Balfour and Ratnieks, 2022). In our case, however, weed communities were dominated by wind-pollinated taxa (including genera *Amaranthus*, *Chenopodium*, *Digitaria* and *Sorghum*), so we only focused on monitoring pollinators on cover crops.

## 2.4. Statistical analysis

Five linear mixed effects models were used to test cover crop biomass production and weed suppression potential. First, we tested the effect of cover crop species and plant status (cover crop vs. weed) and their interaction on plant dry weight, in order to evaluate performance, with field ID and plot ID as nested random factors (Model 1) to account for the potential confounding effects of location. Second, we compared the growth pattern of cover crops and weeds across the season, testing the effect of cover crop species and time (i.e. sampling rounds) and their interaction on either cover crop (Model 2) or weed soil cover (Model 3), once again with field ID and plot ID as nested random factors. Finally, we investigated the importance of germination time on cover crop biomass production and weed suppression with two models testing the effect of crop soil cover in the first sampling round (July) on respectively cover crop (Model 4) and weed dry weight (Model 5). In these last two models, we also added cover crop species as an explanatory variable to take into account intraspecific differences, and field ID was used as a random factor. The dependent variables in all the previously described models were square-root transformed or (in the case of Model 4) log-transformed to meet model assumptions.

Concerning soil quality features, we ran three separate models testing the effects of cover crop species and plant status (cover crop vs. weed) on respectively C/N ratio (Model 6),  $\delta^{13}\text{C}$  (Model 7) and  $\delta^{15}\text{N}$  (Model 8), with field ID and plot ID as nested random factors. In Model 6, we square-root transformed the dependent variable to meet model assumptions.

White mustard achieved abundant flowering in only one of the fields in 2021, as in the others it was overrun by weeds that severely reduced its biomass production and impaired the flowering. Additionally, white mustard was in flower for a much shorter period than buckwheat (see following section). Therefore, we opted to perform statistical analysis only on the much larger buckwheat pollinator visit dataset. In order to evaluate the effect of the sampling period on the number of pollinator visits, we used linear mixed effects models with the number of visits of honeybees (Model 9) and wild pollinators (Model 10) in each plot as the dependent variable, sampling period the explanatory variable and field ID and plot ID as nested random factors. In these models, we also conducted post-hoc tests by calculating pairwise comparisons with a Tukey adjustment. We also calculated linear mixed effects models testing the effect of the number of wild pollinator species on their number of floral visits (Model 11), as well as the effect of number of honeybee visits (a proxy for honeybee density and resource exploitation) on both the number of wild pollinator visits (Model 12) and their species richness (Model 13), with the same nested random factors. To meet model assumptions, the number of wild pollinator visits was square-root transformed in all models in which it was used as a dependent variable.

Finally, we sought to paint a general picture of all the positive ecosystem functions provided by each cover crop species. The original selected indicators of ecosystem functions were total cover crop dry weight (production), total weed biomass control (weed control), mean N/C ratio (nitrogen enrichment), total number of pollinator visits (pollinator visits) and total number of pollinator species (pollinator richness). We used a 0–100% scale, where 100% was the highest value for the corresponding ecosystem function indicator in the dataset. All indicators were set to represent a positive ecosystem function, so we used N/C ratio, the reciprocal of C/N ratio (as it is positively related with

nitrogen content). For the same reason, weed biomass control was calculated according to the formula  $WBC = 100 \cdot (X_{max} - X) / X_{max}$ , where  $X$  is the value of total weed dry weight for a given cover crop and  $X_{max}$  is the highest value of  $X$  in the dataset, once again resulting in a 0–100% scale where 100% correspond to an ideal situation of complete weed control. Finally, we plotted ecosystem function radar charts for each cover crop species.

Analyses were performed using packages nlme v3.1-163, fmsb v0.7.6 and emmeans v1.4.4 (Lenth, 2024) in R v4.3.2 (R Core Team, 2019).

### 3. Results

#### 3.1. Biomass production and weed control

Most cover crops had a highly variable and generally low biomass production and soil cover (Table S4). There was a significant interaction between cover crop species and crop/weed status influencing plant biomass (dry weight) (Table 1, Fig. 1a). High weed biomass values corresponded to low cover crop biomass in each crop species except buckwheat, in which a higher cover crop biomass was coupled with a lower weed biomass (p-value <0.001) (Table S5). Buckwheat cover crop biomass production was also significantly higher than biomass production in other cover crop species (p-value <0.05 – p-value <0.001) (Table S5).

As for growth patterns, there was a significant interaction between time (sampling round) and cover crop species influencing plant soil

**Table 1**

Results of the linear mixed-effects models pertaining cover crop biomass production and weed control, soil quality enhancement potential (2020 data) and pollinator support in buckwheat (2021 data).

Dependent variable	Fixed effects	d.f.	F	p-value
<i>Production and weed control</i>				
Dry weight <sup>a</sup> (model 1)	Cover crop species	5, 63	4.91	<0.001
	Crop/weed status	1, 63	47.88	<0.001
	Species * Status	5, 63	20.41	<0.001
Crop soil cover <sup>a</sup> (model 2)	Cover crop species	5, 57	21.26	<0.001
	Sampling period	2, 105	1.26	0.29
	Species * Period	10, 105	3.54	<0.001
Weed soil cover <sup>a</sup> (model 3)	Cover crop species	5, 63	2.79	0.02
	Sampling period	2, 126	36.08	<0.001
	Species * Period	10, 126	3.21	0.001
Crop dry weight <sup>b</sup> (model 4)	Initial crop soil cover	1, 55	1.53	0.22
	Cover crop species	5, 55	8.29	<0.001
Weed dry weight <sup>a</sup> (model 5)	Initial crop soil cover	1, 54	34.36	<0.001
	Cover crop species	5, 54	3.31	0.01
<i>Soil quality enhancement</i>				
C/N ratio <sup>a</sup> (model 6)	Cover crop species	5, 63	0.69	0.63
	Crop/weed status	1, 39	28.94	<0.001
	Species * Status	5, 39	7.48	<0.001
δ13C (model 7)	Cover crop species	5, 63	1.40	0.24
	Crop/weed status	1, 39	70.72	<0.001
	Species * Status	5, 39	0.22	0.95
δ15N (model 8)	Cover crop species	5, 61	1.30	0.27
	Crop/weed status	1, 35	10.43	<0.01
	Species * Status	5, 35	3.13	0.02
<i>Pollinator support in buckwheat</i>				
Honeybee visits (model 9)	Sampling period	1, 33	15.52	<0.001
Wild pollinator visits <sup>a</sup> (model 10)	Sampling period	1, 33	2.53	0.07
	Wild pollinator species richness	1, 35	70.99	<0.001
Wild pollinator visits <sup>a</sup> (model 11)	Honeybee visits	1, 35	0.37	0.55
Wild pollinator species richness (model 13)	Honeybee visits	1, 35	0.54	0.47

<sup>a</sup> The variable was square-root transformed to meet model assumptions.

<sup>b</sup> The variable was log-transformed to meet model assumptions.

cover for both cover crops and weeds (Table 1). In the case of cover crops, in the initial round both white mustard and especially buckwheat had a significantly higher cover than the other crop types, while in the following rounds it was mostly buckwheat that remained significantly higher than the others (p-value <0.01 – p-value <0.001) (Table S6, Fig. 1b). Conversely, weed soil cover in buckwheat remained relatively low through the sampling period, while in the other cover crop species, weed cover increased through the season (p-value <0.01 – p-value <0.001) (Table S7, Fig. 1c). Additionally, cover crop soil cover during the first round (intended as a proxy for germination time/rate) had no significant effect on final cover crop dry weight (p-value = 0.22), but it had a significant negative effect on final weed dry weight (p-value <0.001) (Table 1, Fig. 1d).

#### 3.2. Soil quality potential

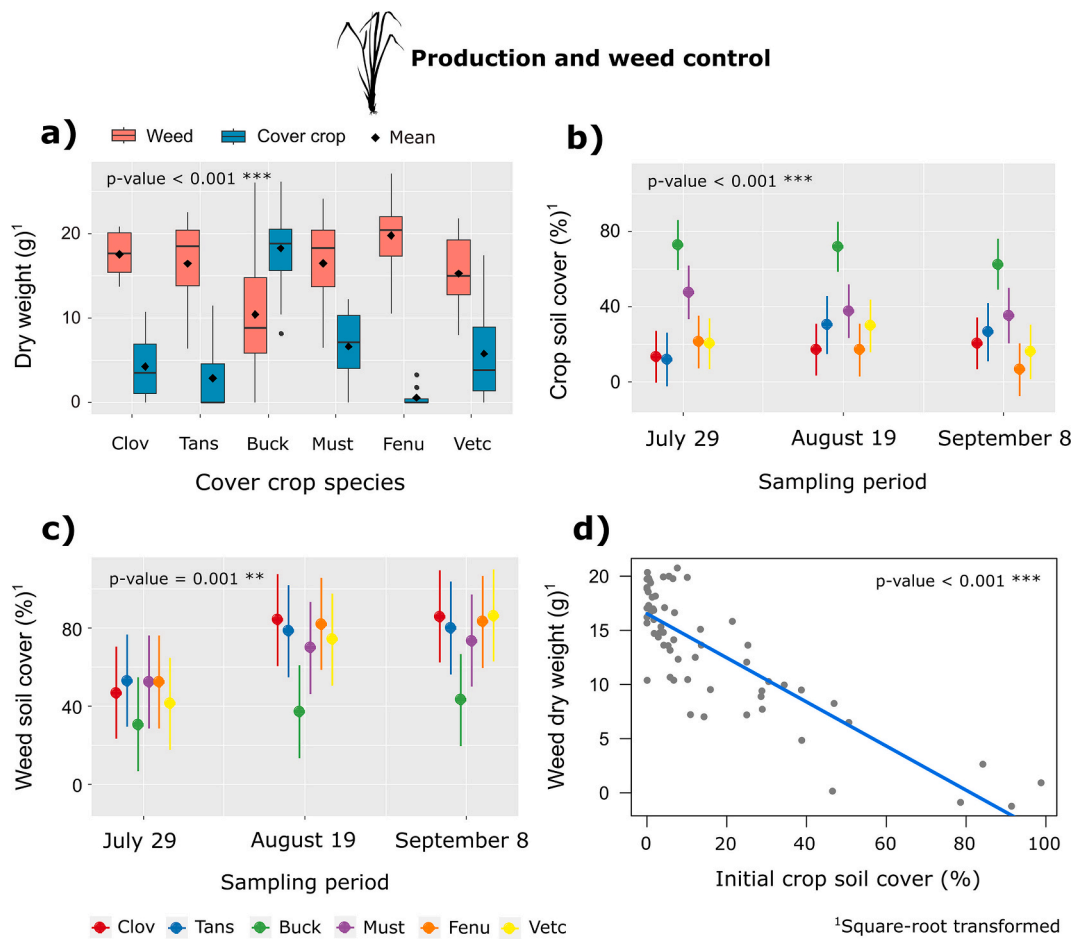
The C/N ratio of plant biomass was significantly influenced by the interaction between cover crop species and cover/weed status (p-value <0.001) (Table 1), being much higher in weeds than in covers for all cover crop types (p-value <0.05 – p-value <0.001) except buckwheat, for which mean C/N ratio in the cover crop was slightly (but not significantly) higher than in weeds (p-value = 0.16) (Table S8, Fig. 2a).

The δ13C was always significantly higher in weeds than in cover crops (p-value = 0.001), irrespective of cover crop species (p-value = 0.95) (Table 1, Table S9). Most cover crop species had a δ15N comparable to weeds (p-value >0.19), with only berseem clover and common vetch having a significantly lower δ15N than weeds (p-value <0.01) (Table 1, Table S10, Fig. 2b).

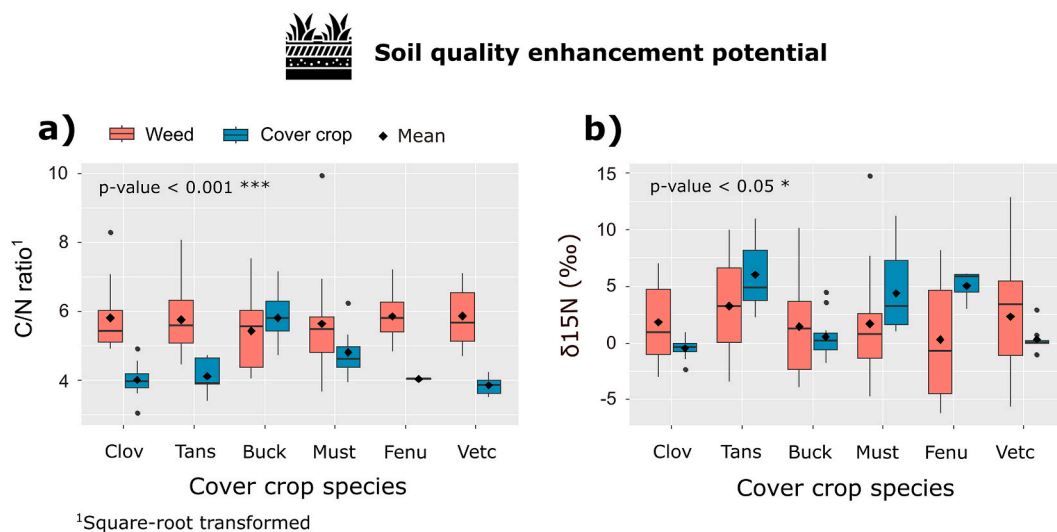
#### 3.3. Pollinator visits

As previously mentioned, during 2020 most cover crops suffered intense competition from weeds and only buckwheat and white mustard reached flowering, so they were the only plants sown in 2021 for the pollinator trial. The vast majority (85.3%) of buckwheat flower visits were performed by honeybees (Table S11). Of the remaining visits, 69.4% were by hoverflies (Diptera: Syrphidae), 11.2% were by wild bee species and 19.4% were by other insects, including beetles, butterflies, moths, flies and wasps. Hoverflies were represented by 15 species and morphospecies, among which the most common were *Eristalis tenax* (L.) (39.9% of hoverfly visits), *Sphaerophoria scripta* (L.) (25.9%) and *Episyrphus balteatus* De Geer (10.8%). Wild bees were represented by 5 morphospecies, with the vast majority of visits (82.6%) being performed by sweat bees (Hymenoptera: Halictidae) and the rest by mining bees (Hymenoptera: Andrenidae). As for the white mustard dataset, it was much more limited, as this species reached full flowering only in one site due to weed competition in the others. Additionally, white mustard remained in full bloom only for the first sampling round, with flowers already partially withered during the second. Honeybees were still the most frequent flower visitors by far on white mustard (91.5%), with an almost negligible number of visits performed by sweat bees (3.5%) and hoverflies (1.5%) (Table S11).

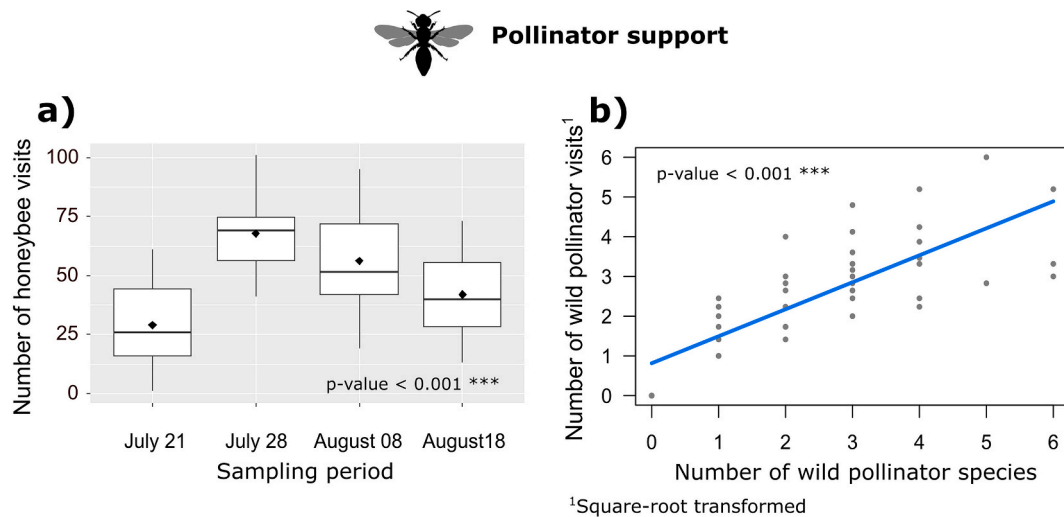
The linear mixed models performed on the buckwheat pollinator dataset revealed more details about pollinator support. Specifically, the sampling period had a significant effect on the number of honeybee flower visits (p-value <0.001), with the last week of July harbouring the highest level of honeybee activity, which was decreasing but remained relatively high by the second week of August (Table 1, Table S12, Fig. 3a). Additionally, the number of wild pollinator flower visits in each plot was significantly positively correlated with the species richness of pollinators visiting the plot (p-value <0.001) (Table 1, Fig. 3b), while there was no significant correlation between the number of honeybee visits and the number of visits from other pollinators (p-value = 0.55), or their species richness (p-value = 0.47) (Table 1).



**Fig. 1.** Plots representing the effects of cover crop species and crop/weed status on dry weight (a), the effects of sampling period and cover crop species on crop (b) and weed soil cover (c), and the effects of initial crop soil cover on weed dry weight (d). Samplings were performed in 2020. Clov = Berseem clover; Tans = Blue tansy; Buck = Buckwheat; Must = White mustard; Fenu = Fenugreek; Vetc = Common vetch. Error bars indicate the confidence intervals (95 %).



**Fig. 2.** Plots representing the effects of cover crop species and crop/weed status on C/N ratio (a) and  $\delta^{15}N$  (b). Samplings were performed in 2020. Clov = Berseem clover; Tans = Blue tansy; Buck = Buckwheat; Must = White mustard; Fenu = Fenugreek; Vetc = Common vetch.



**Fig. 3.** Plots representing the effects of sampling period on honeybee flower visits (a) and the effects of wild pollinator species richness on their number of flower visits (b). Samplings were performed in 2021 on buckwheat.

## 4. Discussion

### 4.1. Biomass production, weed control and soil quality

The biomass production of buckwheat was remarkably higher than the other tested species (Table 1, Fig. 1a), implying that buckwheat likely has an edge in terms of its green mulch functionality and seed production (Ruis et al., 2019). Considering both biomass and soil cover, buckwheat was also the most promising cover crop for weed control (Table 1). This species grew rapidly after germination, keeping a high soil cover from the beginning of the sampling season to the harvest (Fig. 1b). This is likely one of the main reasons why, in the studied context, buckwheat proved to be far more competitive against weeds than the other tested cover crop species, which resulted in a significantly lower weed soil cover across the season, and a lower final weed dry biomass (Fig. 1c and d). As evidenced by Model 5, early and abundant soil covering might in fact be a decisive element in making buckwheat so successful in weed suppression, a conclusion that stands in line with other reported studies (Brust et al., 2014; Mirsky et al., 2011). Aside from the fast germination and high growth rate, the success of buckwheat over weeds might also be explained by allelopathic effects, which have been described before for this species (Bulan et al., 2015; Iqbal et al., 2003). White mustard also had a higher early soil cover than most other tested cover crops (Fig. 1b), but it proved itself to be less competitive against weeds, as its overall soil cover was significantly lower than buckwheat, and not nearly as efficient in limiting weed soil cover or biomass production, with white mustard biomass also being significantly lower than buckwheat biomass (Fig. 1a). The other tested crops failed to contain weed spread, probably mainly due to a late and/or low rate of germination and growth during the first stages of vegetation development, further hinting that these factors are crucial for rapid cover of the soil before weeds become more competitive (Mirsky et al., 2011). The legume species (i.e. common vetch, fenugreek, and berseem clover) exhibited a very low weed control function. Legumes have been proven to be effective weed suppressor when used in mixtures and in long term experiments, also by enhancing soil properties (Alonso-Ayuso et al., 2018; Malaspina et al., 2023). For instance, common vetch showed a particular potential for long-term weed management, also due to biochemical inhibitory effects on root growth of some weed species (Adeux et al., 2021; Kunz et al., 2016; Rueda-Ayala et al., 2015). It is hence plausible that our experimental conditions were not suitable to express the full potential of some cover species. The use of mixed cover crops including legumes for long-term weed management might

thus merit additional research. Additionally, by outcompeting weeds, cover crops can also influence weed seed bank composition in the long term (Alonso-Ayuso et al., 2018) and, in some cases, even reduce seed bank density in the years following the start of their employment (Moonen and Bàrberi, 2004; Schmidt et al., 2019). The potential of buckwheat, as well as cover mixtures, for long-term weed reduction also through seed bank reduction will thus deserve to be further investigated.

In terms of soil fertility enhancement potential, cover crop contribution was very different. The carbon-to-nitrogen ratio is known as a reliable indicator to evaluate the balance between two elements essential for crop growth and microbial health, i.e. C and N (van der Sloot et al., 2022). Cover crops, and especially legumes, are expected to have low C/N ratios, and their biomass decomposes relatively quickly because the C content is offset by adequate amounts of N (Finney et al., 2016; Ranells and Waggener, 1996). In this light, we expected our selected species to show lower C/N ratio than weed community, potentially enhancing the organic matter quality added to the soil. Our findings suggest that buckwheat is likely the least proficient of the tested cover crops at enriching soil with nitrogen, with blue tansy, fenugreek and especially berseem clover and common vetch faring much better for the contribution to soil organic matter quality (low C/N ratio - Fig. 2a). Nonetheless, some authors pointed out that intermediate values of C/N ratio are also suitable for a good organic matter added to soil, in relation to the rate of N mineralization and leaching (Flavel and Murphy, 2006; Watson et al., 2002).

Atmospheric N has a lower  $\delta^{15}\text{N}$  signature than N of agricultural soils, and plants capable of atmospheric nitrogen fixation (e.g. legumes) exhibit a diluted  $\delta^{15}\text{N}$  compared with the soil (Cox et al., 2022; Munroe and Isaac, 2013). Our results confirmed that the selected legume species (i.e. common vetch, fenugreek, and berseem clover) exhibited consistent low C/N and  $\delta^{15}\text{N}$  values (Tables S8–S10, Fig. 2b), that could hence be used as indicator of efficiency in atmospheric nitrogen fixation (Gentili and Huss-Danell, 2019).

As for  $\delta^{13}\text{C}$ , no cover crop species was significantly different from the others and, in fact, they all had a significantly lower  $\delta^{13}\text{C}$  than weeds (Table S9). This also suggests that all tested cover crops are at a disadvantage against weeds in terms of water stress resistance, an element that might have played a key role in the poor performance of many species, while species such as buckwheat apparently rely on additional adaptations (including fast germination, growth rate and allelopathic effects) that compensate for this weakness in terms of final production and weed control. In relation to these conditions, buckwheat also exhibited a relatively low  $\delta^{15}\text{N}$  value that could be related to the

adsorption of mineral N from deeper soil layers or under soil drought conditions (Groß-Schmolders et al., 2022). In addition, many weeds occurring in the experimental sites (e.g. *Amaranthus* spp., *Digitaria sanguinalis* (L.), *Sorghum halepense* (L.) Pers.) have C4 carbon fixation, which allows them to better cope with high temperatures and drought conditions. Generally, C3 plants have low  $\delta^{13}\text{C}$  ( $-24$  to  $-34\%$ ) while C4 plants have high  $\delta^{13}\text{C}$  values ( $-6$  to  $-19\%$ ), which is consistent with our values (Table S4). This difference in stable carbon isotope has been already used as a tracer for the labelling of soil organic matter (Mariotti and Balesdent, 1990; Martin et al., 1990; Park et al., 2023).

#### 4.2. Support to pollinators

Before discussing pollinator results, it is worth mentioning some limitations related to the single season monitoring. Pollinator populations are known to fluctuate and, in some cases, follow long-term trends (Petanidou et al., 2008; Potts et al., 2010), which our study is of course unfit to reveal. Such changes would require a minimum of 5 years of data collection to be properly detected and understood (Aldercotte et al., 2022), but this was outside the scope of our study, which was focused on cover crops as a support to pollinators. For such shorter-term investigations, sampling campaigns limited to one year are not uncommon (Campbell et al., 2017; Feltham et al., 2015), and are generally thought to yield valuable data, as long as the sampling is carried out in multiple sites representative of the study area, repeatedly across the season, and in optimal weather for pollinators – all conditions that we met.

As the only species able to overcome weed competition to the point of reaching abundant flowering in all the study sites, buckwheat emerged among the tested species as the best cover crop for supporting pollinators (Table S11). Honeybees are especially likely to benefit from this crop, as they were by far the most frequent flower visitors – a dominance that is in line (Bjorkman, 1995) or even more pronounced (Jacquemart et al., 2007; Liu et al., 2020) than the results from other geographical areas, which frequently report honeybee as the main buckwheat pollinator. Additionally, the significant increase in honeybee visits at the end of July and the beginning of August (Fig. 3a) suggests that this is the period in which these insects require more support in terms of floral resources, likely because of the lower availability of wildflowers (Balfour et al., 2018; Timberlake et al., 2019). Buckwheat, which in the study area has a long flowering period ranging from the second half of July to the end of August or the beginning of September, can provide abundant nectar resources during this critical time.

As for wild pollinators, in the study area buckwheat was mostly visited by hoverflies (Table S11). Hoverflies are prized not only for their importance as pollinators (Doyle et al., 2020; Hodgkiss et al., 2018), but also for the fact that the larvae of many species can act as efficient biocontrol agents of aphids (Pekas et al., 2020; Wotton et al., 2019). These predators include some of the most common species we recorded in our study, such as *S. scripta* (Gojković et al., 2020) and *E. balteatus* (Leroy et al., 2010) (Table S11). Buckwheat thus has the potential to enhance both pollination and pest control (van Rijn et al., 2013).

It is also worth remarking on the fact that wild pollinator species richness was positively linked with the number of flower visits (Table 1, Fig. 3b), further confirming the importance of protecting pollinator biodiversity to maintain pollination services (Brittain et al., 2013; Hoehn et al., 2008; Klein et al., 2003). In this regard, a positive note might be represented by the lack of correlation between the number of honeybee visits and the number of visits and species richness of wild pollinators (Table 1). This might mean that the use of buckwheat in the study area can benefit honeybees and, in part, wild pollinators without promoting negative effects of the former on the latter through competition, which is not always a given according to the available literature (Magrath et al., 2017; Ropars et al., 2019). Recent findings (Fijen et al., 2022) do indeed suggest that, by taking some precautions (such as limiting the density of honeybee hives in the area), buckwheat

cultivation can support both wild and managed pollinators without promoting competition.

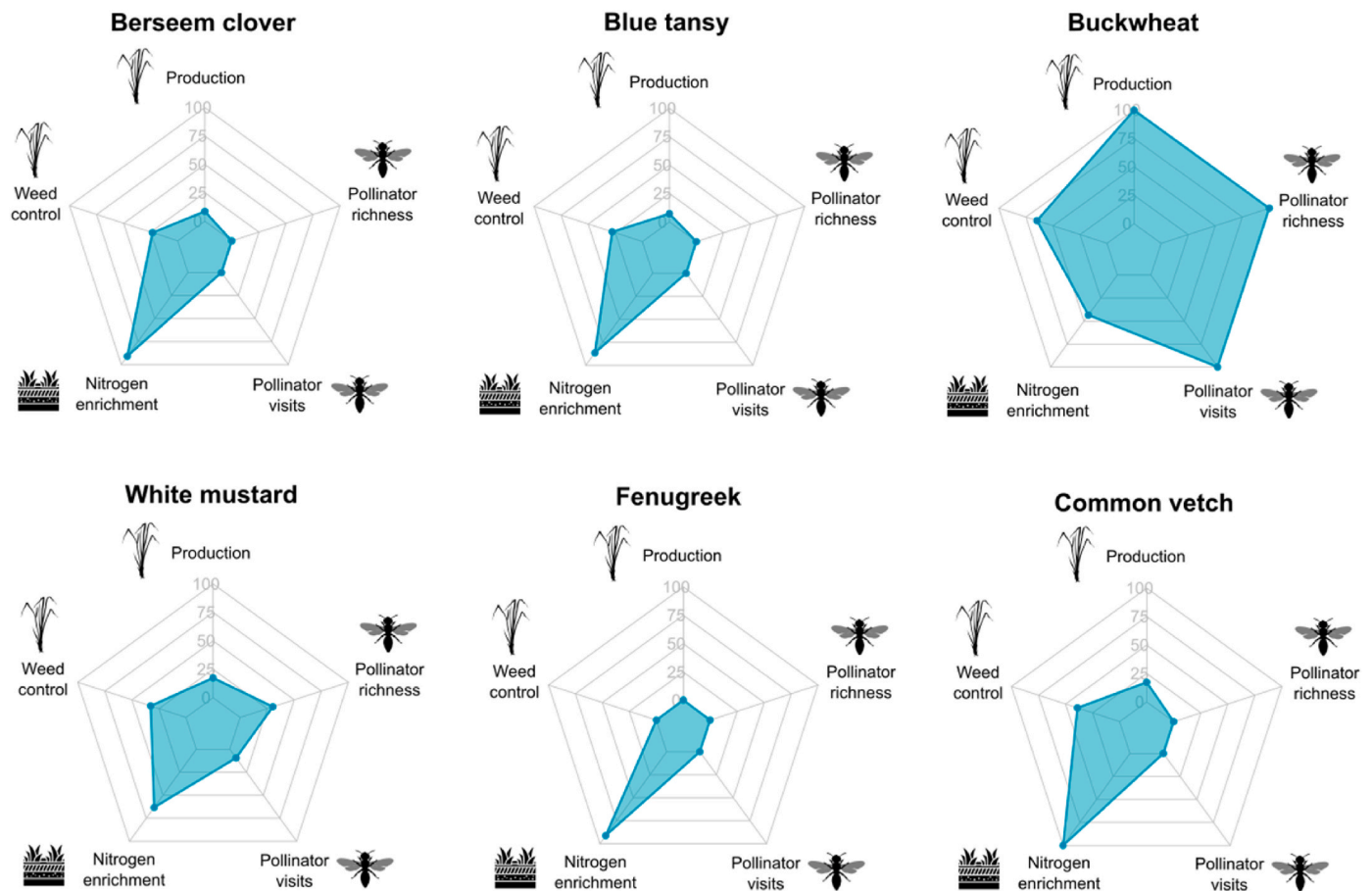
It should also be noted, however, that the number of wild pollinator visits on buckwheat was generally low if compared with honeybees (Table S11). Wild bee visits, in particular, were only a tiny minority of the total. Wild pollinator visits were even lower in the (admittedly much more limited) white mustard dataset. Wild insect species are hugely important for pollination services (Breeze et al., 2011; Garibaldi et al., 2013, 2014) and, in this respect, the largest part is played by wild bees (Goulson, 2003; Lowenstein et al., 2015), which tend to be more efficient pollinators than hoverflies and other groups (Jauker et al., 2012; Nabhan and Buchmann, 1997). Future research should therefore focus on identifying flowering cover crop species that are attractive to a wider variety of wild pollinators in general and wild bees in particular, and can thus be used to complement buckwheat (Mallinger et al., 2019) in conserving insect biodiversity and promoting pollination services.

#### 4.3. Overall cover crop performance

Our analysis of multiple ecosystem functions identified buckwheat as the most promising and best performing of the tested summer cover crop species overall (Fig. 4). Being the fastest growing and most competitive species, buckwheat was not only able to control weeds, but also yielded a higher biomass production and soil cover (and thus soil protection), while also reaching flowering and providing abundant resources for honeybees and a fraction of wild pollinators during a critical period of the year. The analysis however also revealed that buckwheat also had some weaknesses; namely, its potential for soil nutrient enhancement was low if compared with other species, and its floral resources, while abundantly exploited by honeybees and hoverflies, were much less attractive for wild bee species. This underlines the necessity of finding other complementary cover crops that could be used alongside buckwheat in a mixture to cover these aspects. Nitrogen-fixing legumes are the most promising plants in terms of soil nutrient enrichment (Smith et al., 1987). Legumes could simultaneously improve the range of the support to pollinator communities, as their flowers represent an important food source for bumblebees and other wild bees (Cole et al., 2022). Legume species tested in this study were mostly overcome by weeds before producing a significant amount of biomass and reaching the flowering stage, so more competitive, short-lived species should be selected. A key feature in this regard might be represented by resistance to water stress. All cover crops in this study were put under significantly higher water stress if compared with the competing weeds, and while this did not prevent buckwheat from controlling weeds, it might be a decisive element for other cover crops with a different biology (Patterson, 1995).

## 5. Conclusions

Our research showed that focusing on multiple functions simultaneously is a sound strategy for the evaluation and selection of summer cover crop species capable of improving a variety of agroecosystem aspects. Weed suppression abilities are a highly desirable trait, not only because weed suppression itself is a pivotal ecosystem function, but also because cover crops able to outcompete weeds are also the most likely to reach a phenological stage in which they can perform additional functions. In our case, for instance, buckwheat was the best cover crop for weed control, which allowed it to also outperform the others in terms of biomass production and support to pollinators (especially honeybees and hoverflies). Our approach also allowed us to highlight the weaknesses of the tested cover crop species and, therefore, the traits that would be needed in complementary species. Specifically, buckwheat is lacking in terms of soil enhancement potential and support to wild bee species; its complementary cover crops should therefore perform these two functions, and also share the ability of buckwheat to outcompete weeds either through fast germination and growth, allelopathic effects



**Fig. 4.** Radar charts summarizing the performances of each tested cover crop species in the main ecosystem functions considered. All indicators were transformed in order to represent a positive ecosystem function and to use a 0–100% scale, where 100% was the highest value for the ecosystem function in the dataset. Indicators were cover crop dry weight (production), weed dry weight control (weed control), N/C ratio (nitrogen enrichment), number of pollinator visits (pollinator visits) and number of pollinator species (pollinator richness).

or other adaptations. This is particularly true for the selection of summer cover crops, when climate conditions trigger fast spread of weeds on bare soil. Our results evidence that selecting species with a prompt germination and fast growth might be crucial. Given their ecology, this would narrow the search to highly competitive and fast-growing legume species, and groups that share their ecological features. Our results could improve cover crop selection schemes, suggesting the necessity for a comprehensive approach focusing on agroecosystem multifunctionality.

#### CRediT authorship contribution statement

**Francesco Lami:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marco Vuerich:** Writing – review & editing, Investigation. **Michele Fabro:** Writing – review & editing, Resources, Funding acquisition. **Pietro Zandigiaco:** Writing – review & editing, Validation. **Enrico Braidot:** Writing – review & editing, Validation. **Elisa Petrusa:** Writing – review & editing, Validation, Supervision. **Stefano Barbieri:** Writing – review & editing, Resources, Funding acquisition. **Valentino Volpe:** Writing – review & editing, Validation. **Maurizia Sigura:** Writing – review & editing, Supervision, Investigation. **Gemini Delle Vedove:** Writing – review & editing, Validation. **Francesco Boscutti:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cropro.2024.106832>.



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