Soil texture involvement in wildflower strip ecosystem services delivery in Mediterranean agro-environment

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A B S T R A C T

The biodiversity restoration of the agroecosystem through wildflower strips is one of the most important agronomic strategies of the last few decades. However, poor knowledge of the bio-agronomic characteristics of the various wildflower species often leads to suboptimal performances especially as a result of their poor seed germination and poor seedling emergence due to the erratic soil texture. A two-year experiment involving the sowing of eighteen wildflower species in different texture conditions highlighted how some species emerged earlier or more effectively than others and verifying the consequent ecosystem service provided to pollinators. Species with smaller seeds were more inhibited by sowing in soil with high content of clay. Additionally, an inverse relationship (Boltzmann's sigmoidal regression) was found between seed weight and soil-mediated inhibition. This soil-mediated inhibition was higher in the clayey textured soil, showing that this inhibition was mediated both by seed weight and by the clay particles of the soil. The reciprocal interference between the emerged seedlings also determined a further decline in the wildflower species with small seeds. This differing agronomic performance, after sowing in the two different soil textures, did not affect the number of pollinator visits. Performance differed both in terms of floristic biodiversity and pollinator biodiversity. Finally, the experiment highlighted the importance of the appropriate selection of the wildflower species for biodiversity restoration - not only in terms of pollen and nectar availability for pollinators, but also in terms of compatibility with the soil texture in which the wildflower seeds are sown.

1. Introduction

One of the most important agronomic challenges of the new millennium is the development of agronomic strategies capable of maintaining the ecosystem services provided by pollinators. Biodiversity is important not only from an ecological point of view but also in terms of agroecosystem productivity, especially for insect-pollinated crops (Montoya et al., 2019). Unfortunately, in recent decades there has been an increasing decline in pollinators in terms of both quantity (overall number of bees, Potts et al., 2010) and biodiversity (Millard et al., 2021). Although the economic damage is not easy to quantify, it has been estimated that this decline could have a strong agronomic impact in terms of long-term agricultural sustainability (Bauer and Wing, 2016).

Although the causes are not entirely clear, the increasing crop intensification certainly plays a key role in decreasing the biodiversity of the agroecosystem (Tscharntke et al., 2012). For example, conventional cropping systems are typically poor overall in weed biodiversity in terms of wildflowers (Bretagnolle and Gaba, 2015) as a consequence of the strong and unsustainable agronomic pressure (Goulson et al., 2015). In fact, wildflower weeds were once abundant and clearly visible in rural landscapes thanks to the corollas having evolved towards their "eye catching" role in order to attract pollinators (Schiestl and Johnson, 2013).

Unfortunately, this intensification in cultivation has above all reduced the insect-pollinated weeds, thus reducing the availability of pollen and nectar necessary for the survival of pollinators. The ancient rural landscapes, which were once rich in wildflower-weeds (Twerski et al., 2022), are being depleted the weed biodiversity that constituted an ecosystem service for the survival of pollinators.

There are many agronomic strategies aimed at restoring plant-pollinator biodiversity, such as the planning of field margins (Morrison et al., 2017), hedgerows (Clausen et al., 2022), uncropped areas (Holland et al., 2015), a mosaic management of agricultural landscape and wildlife habitats (Hall et al., 2022), and finally by sowing wildflower strips (Haaland et al., 2011).

Wildflower strips not only benefit bees and other pollinators (Ganser et al., 2021), which increase crop pollination (Delphia et al., 2022), but also constitute a vast ecosystem service (Sutter et al., 2018). Wildflower strips also benefit the sustainability of crop productivity (Tschumi et al., 2016) and protection (Mei et al., 2021), thanks to the diffusion of pollinators that are predators (larval stage) of harmful insects such as the

aphidophagous hoverflies (Albrecht et al., 2021).

The ecosystem services provided by wildflower strips are effective especially if they are integrated within a mosaic agricultural landscape (Hellwig et al., 2022) in a surrounding agro-environment which is lacking in blooms (Schubert et al., 2022). Unfortunately, obtaining a good agronomic performance through wildflower sowing is not easy since often the species that become established are only a minority of those present in the seed mixture (Stroot et al., 2022). In fact, the sowing success strictly depends both on the seed quality (Schmidt et al., 2022) and the competitiveness of each species against the pre-existing weed flora (Benvenuti and Bretzel, 2017).

However, the greatest problem arises from the poor ability of many wildflower species to germinate and emerge in erratic soils, especially in terms of texture, and in contrast to fertile soils (Schmidt et al., 2020). Following the framework of the EU Common Agricultural Policy, it is common for farmers to allocate these non-productive "ecological focus areas" to less fertile soils, often with a silty-clayey texture that tend towards compaction.

In practice, the choice of seed mixture should be based not only on the ability to produce pollen and nectar for pollinators, but also on its ability to germinate and emerge in erratic soil textures. Seed weight plays a crucial role in terms of compatibility with certain soil textures. Previous experiments aimed at forcing the germination of the weed seed bank through a stale seedbed preparation have shown that the smallest seeds often remain dormant within the soil clods generated by the clay particles (Benvenuti et al., 2021). This soil-imposed dormancy is due to the restricted gas diffusion of clayey particles, which limits the removal of toxic volatile metabolites (acetaldehyde, acetone and ethanol) generated by the typical hypoxic conditions of buried seeds (Benvenuti, 2003). Therefore it is possible that wildflower species with smaller seeds are more difficult to cultivate in clayey soils and therefore require soils characterized by a sandy matrix.

We conducted a two-year series of experiments in order to: i) investigate the relationship between soil texture and seed weight in terms of seed germination and seedling emergence, and ii) verify their agronomic performance in terms of both the biodiversity of wildflowers and pollinators from the perspective of ecological restoration in an agricultural environment.

2. Materials and methods

2.1. Plant material

Floristic investigations carried out in the last decade have identified various wildflower communities (Table 1) in diversified agro-environments in Tuscany (central Italy) that are widely visited by pollinating insects. The species were selected for the aesthetic impact of their highly chromatic corollas (Fig. 1) and their mutualistic link with pollinators. In summer 2020, seeds of all the eighteen wildflower species were collected from the relative senescent plants and were transferred to the laboratory, where they were cleaned and stored at room temperature until their experimental use.

Seed weight (or fruit as in the case of the achenes of Asteraceae and Apiaceae) was determined by weighing 1000 seeds chosen randomly according to ISTA rules for seed testing (ISTA, 1999).

2.2. Wildflower sowing

All the wildflowers were then sown in 2021 and 2022 (0.5–1 cm depth), in mid-February of both years, in large containers (1 ×1 m, 1.2 height) filled with sandy-loam (sand 74.8%, silt 18.6%, clay 6.6, pH 7.2, organic matter 1.9%) or clay-loam (sand 34.4%, silt 35.3%, clay 30.3, pH 7.4, organic matter 2.1%) textured soils. Both experimental soils were in optimal conditions of soil structure. The containers were placed in the open air on the experimental farm of the Agronomy Department of Pisa University (43°70'N, 10°43'E).

Table 1

Botanical data (Pignatti, 1982) and seed collection environments of the tested wildflowers.

Wildflower Species	Botanic Family	1000 Seed Weight (g)	Environment of seed collection	Chorology
Agrostemma githago L.	Cariophyllaceae	12.02	Agricultural fields	Eurosiber.
Anacyclus radiatus Loisel.	Asteraceae	0.10	Field margins	Steno-Medit
Anthemis mixta L.	Asteraceae	0.44	Arid grasslands	Euro-Asiat.
Blackstonia perfoliata (L.) Huds.	Cariophyllaceae	0.01	Arid grassland	Euri-Medit.
Centaurea cyanus L.	Asteraceae	4.36	Agricultural fields	Steno-Medit.
Centaurium erythraea Rafn.	Gentianaceae	0.01	Arid grasslands	Paleotemp.
Chrysanthemum coronarium L.	Caryophyllaceae	2.84	Fields margins	Steno-Medit.
Chrysanthemum segetum L.	Asteraceae	1.42	Agricultural fields	Steno-Medit.
Consolida regalis Gray	Ranunculaceae	1.52	Agricultural fields	Euri-Medit.
Lavatera punctata All.	Malvaceae	5.34	Field margins	Steno-Medit.
Legousia speculum-veneris (L.) Chaix	Campanulaceae	0.16	Agricultural fields	Euri-Medit.
Myosotis arvensis (L.) Hill	Boraginaceae	0.48	Arid grasslands	Euro-Asiat.
Nigella damascena L.	Ranunculaceae	2.18	Agricultural fields	Euri-Medit.
Orlaya grandiflora (L.) Hoffm.	Apiaceae	11.07	Arid grasslands	Euro-Asiat.
Papaver rhoeas L.	Papaveraceae	0.09	Agricultural fields	Euri-Medit.
Silene conica L.	Caryophyllaceae	0.38	Arid grasslands	Paleotemp.
Tripleurospermum inodorum (L.) SB	Asteraceae	0.35	Field margins	Eurosiber.
Vaccaria hispanica (Mill.) Raushert	Caryophyllaceae	5.02	Field margins	W-Asiat.

The seed dose was standardized to a density of approximately 150 plants m-2 (ranging from 0.1 to 10 g m-2 according to the size and seed weight of the different wildflower species, Fig. 2) in order to obtain a balance between the eighteen selected species. The seed dose was calculated, for each species, in a directly proportional way to their 1000 seed weight and inversely to their germinability (Bretzel et al., 2012) preliminarily evaluated in the laboratory in Petri dishes incubated in climatic chamber regulated at a temperature of 20 °C with a photoperiod of 12/12 (light/dark respectively). After seeding, light rolling was applied in order to facilitate the seed-soil contact. Each type of soil texture (sandy-loam or clay-loam) was replicated ten times for a total of twenty containers.

2.3. Seed germination, seedling emergence and calculation of soil-mediated inhibition

In order to calculate the soil-mediated emergence-inhibition, seeds were incubated in Petri dishes to evaluate their germination in optimal conditions without soil. Seeds of each wildflower species were placed in Petri dishes (9 cm diameter equipped with Whatman filter paper No. 1, 100 seeds each) suitably moistened with distilled water. The Petri dishes were wrapped in a double layer of aluminium foil, to avoid direct exposure to the sun's rays. They were placed, on the same day on the surface of additional containers identical to those described in Section 2.2. This method enabled the seeds to incubate in the same temperature conditions as the seeds sown into the two different soil textures.

Fig. 1. Flowers of the eighteen tested wildflowers species: 1 = *A. githago*. 2 = *A. radiatus*. 3 = *A. mixta*. 4 = *B. perfoliata*. 5 = *C. cyanus*. 6 = *C. erythraea*. 7 = *G. coronarium*. 8 = *G. segetum*. 9 = *C. regalis*. 10 = *L. punctata*. 11 = *L. speculum-veneris*. 12 = *M. arvensis*. 13 = *N. damascena*. 14 = *O. Grandiflora*. 15 = *P. rhoeas*. 16 = *S. conica*. 17 = *T. inodorum* and 18 = *V. hispanica*.

Fig. 2. Seeds of the eighteen tested wildflowers species: 1 = *A. githago*. 2 = *A. radiatus*. 3 = *A. mixta*. 4 = *B. perfoliata*. 5 = *C. cyanus*. 6 = *C. erythraea*. 7 = *G. coronarium*. 8 = *G. segetum*. 9 = *C. regalis*. 10 = *L. punctata*. 11 = *L. speculum-veneris*. 12 = *M. arvensis*. 13 = *N. damascena*. 14 = *O. Grandiflora*. 15 = *P. rhoeas*. 16 = *S. conica*. 17 = *T. inodorum* and 18 = *V. hispanica*. Vertical bars indicate 1 mm.

After 2–3 weeks of seed incubation, the germination percentage (cotyledon appearance) of each species was analysed. Experiments were stopped after three weeks when no further emergence (one week after the last) was observed. Each species was replicated three times for a total of 54 Petri dishes (three replicates x 18 wildflower species) using a completely randomized design.

The evaluation of seedling emergence was carried out in both years (2021 and 2022) one month after sowing in mid-March. During this period there were rainfalls with a low degree of intensity (overall of 25 and 38 mm in 2021 and 2022 respectively) and consequently there were no problems due to the formation of surface crust. This seedling count was carried out within a square frame (25 cm) randomly arranged on the container surfaces. Five counts were carried out in each container.

The emergence inhibition, compared to optimal laboratory conditions, was calculated as a percentage of the difference between Petri dish germination and seedling emergence in sandy-loam or lay-loam soil. In addition the percentage of soil-inhibition was plotted with the 1000 seed weight of each wildflower species using the regression that best describes the relationship between seed weight and soil-mediated emergence inhibition.

2.4. Plant density at the flowering phenological stage

The plant density during full flowering was evaluated in both years

(2021 and 2022) in mid-May. The plant count was carried out within a square frame (25 cm, divisible in two parts to be placed in the dense vegetation canopy) randomly arranged in the surfaces of the experimental containers. Five counts in each container were carried out. Data were expressed as a percentage of plant density decreasing at the phenological stage of flowering with respect to the initial seedling density detected one month after sowing.

2.5. Flowering dynamics

For each container, five plants of each of the eighteen wildflower species were marked with paper labels to evaluate the flowering dynamics. For each species 30 plants were used. Data, collected weekly, were expressed as flowering time during the experimental period.

2.6. Evaluation of flower visitors

Pollinator biodiversity was evaluated in May, since this is the period when the flowering of all species occurs. On two days each week, in each container of both soil textures, the pollinator count was carried out at the following times: morning (from 10:00–12:00) and early afternoon (from 14:00–16:00). Data were collected by placing a 0.5×0.5 m plastic frame at the center of each container (1 m x 1 m). Pollinators landing on the flowers inside this area were counted during foraging, noting the respective categories: bees, solitary bees, bumblebees, flies (hoverflies, bee flies), and butterflies.

Observations lasted five minutes for each of the 20 plots (2 soil textures x 10 replicates). From these records, the percentage visits of the different pollinator categories were calculated. Data were expressed both as the number of total visits m^{-2} h^{-1} and as percentages (with respect to the total) of the above mentioned categories.

2.7. Calculation of wildflower and pollinator diversity

Data on the wildflower community and relative pollinator were used to calculate the diversity indexes. The Shannon diversity index (H'), was used as follows:

$$H' = - \sum_{i=1}^{k} p_i \log p_i$$

where k is the number of wildflower species or pollinator groups, and p_i is the fraction of individuals belonging to the ith each wildflower community or pollinator group.

2.8. Statistical analyses

For all the experiments (both laboratory and field experiments), a completely randomised experimental design (three replicates) was adopted. Data were pooled over the two experimental years because there was no significant interaction (means analysed by one-way ANOVA, $p < 0.05$) between 2021 and 2022.

The variables analysed were: i) seedling emergence-inhibition, ii) wildflower density, iii) total number of visitors (flowers^{-1} h^{-1}), iv) wildflower biodiversity, and v) pollinator biodiversity among the different categories (bees, solitary bees, bumblebees, diptera syrphidae, diptera bombyliidae and Lepidoptera).

After the homogeneity test of variance, using the Kolmogorov-Smirnov D test and the Cochran test, respectively, arcsine transformation of percentage data was carried out to normalize the data distribution (Steel and Torrie, 1980). Angular values (seedling emergence, soil inhibition and pollinator groups) and untransformed data (plant density, total flower visitors, wildflower biodiversity and pollinator biodiversity) were subjected to ANOVA using Duncan's multiple range test ($p < 0.05$ and/or $p < 0.01$) for mean separation (least-significant difference, LSD).

Finally, the seedling emergence and soil-inhibition values of the eighteen wildflower species were plotted with the corresponding 1,000-seed weight and fitted with a Boltzmann sigmoidal regression. For the statistical analysis, CoHort software (CoStat, Monterey, CA, USA) was used.

3. Results

3.1. Seed weight, germination and seedling emergence

The 1000 seed weight of the wildflower species tested was very diverse (Table 1), ranging from 0.01 g of *Blackstonia perfoliata* (L.) Huds. and *Centaurium erythraea* Rafn. (both Gentianaceae) to 12.02 g of *Agrostemma githago* L. and 11.07 of *Orlaya grandiflora* (L.) Hoffm. (Cariophyllaceae and Apiaceae, respectively). Most of the species showed a very light seed weight. Exactly half showed a 1000 seed weight less than 1 g. Their germinability in optimal conditions (Petri dishes) was also very variable (Table 2), ranging from rather poor shown (*Consolida regalis* Gray, 44.3%) to very high (*A. githago,* 95.2%). Overall one third of the species showed a germination of less than 60% and only five species exceeded 80%.

The sowing of the various wildflower species in the soil matrix showed, albeit to a very different extent, a reduction in their seed germination and seedling emergence. This reduction, already substantial in the case of sandy-loam soil, was basically more marked and statistically significant ($p < 0.05$) in the clay-loam textured soil. The average temperatures recorded during the emergence tests (2021–2022 two-year period) were respectively 4–10 °C in February and 5–15 °C in months of March (average of minimum and maximum respectively). More than half of the tested species (*Anacyclus radiates* Loisel., *Anthemis mixta* L., *B. perfoliata*, *C. erythrea*, *Crysanthemum coronarium* L., *Crysanthemum segetum* L., *C. regalis*, *Legousia speculum-veneris (L.) Chaix*, *Myosotis arvensis* (L.) Hill, *Papaver rhoeas* L., *Silene conica* L. and *Tripleurospermum inodorum* (L.) SB) showed a decrease in seed germination and seedling emergence (statistically significant between each of the three incubation conditions, $p < 0.05$) from soilless conditions (Petri dishes), to sandy-loam or clay-loam soil sowing. Each of these twelve species showed a highly different degree of inhibition (statistically significant, $p < 0.01$) after sowing in the two different soil textures. In contrast, the remaining six species (*A. githago*, *Centaurea cyanus* L., *Lavatera punctate* All., *Nigella damascena* L., *O. grandiflora* and *Vaccaria hispanica* (Mill.) Raushert) showed no statistically significant emergence-inhibition in the comparison between the two different soil textures.

Fig. 3 shows a Boltzmann sigmoidal regression (statistically significant $p < 0.05$) between the 1000 seed weight of the eighteen tested wildflowers and the respective soil emergence-inhibition induced by both soil types (expressed as the emergence percentage with respect to the germination performance detected in the Petri dishes). As the figure shows, the lighter seeds showed a greater soil-mediated emergence-inhibition. This inhibition was decidedly higher in the soil characterized by smaller particles (Fig. 3A), but was less marked in the characterized by larger particles (Fig. 3B).

3.2. Wildflower density and flowering dynamics

As expected, there was a strong interference among the emerged seedlings of the eighteen species (Fig. 4). In fact both wildflower communities grown in the different soil textures decreased in density (Table 3) between mid-March and mid-May. However, this decrease was higher and statistically significant ($p < 0.05$) in the clay-loam soil compared to the sandy-loam soil. However, while some species characterized by relatively large and heavy seeds (*A. githago*, *C. cyanus* and *L. punctata*) showed no mortality during growth, other species with small and light seeds (i.e. *A. mixta*, *B. perfoliata*, *C. erythraea*, *L. speculum-veneris, M. arvensis, P. rhoeas, S. conica* and *T. inodorum*) showed a non-

Table 2

Germination in laboratory conditions and seedling emergence in both soil textures of the eighteen wildflower species. Means of the germination and soil emergence performances (within second, third and fourth columns from the left) followed by different letters show statistical difference (Duncan's test) for p < 0.05. The soil inhibition (as reduction % to respect to Petri dish germination) of the two soil textures and relative statistical significance between them (n.s.= not significant, *= p < 0.05, **= p < 0.01) are reported.

Wildflower species	Germination in Petri dish	Soil emergence (%)		Soil inhibition[1] (%)		Statistical Significance[2]
		Sandy-loam	Clay-loam	Sandy-loam	Clay-loam	
Agrostemma githago L.	95.2 a	94.1 a	90.3 a	1.2	5.1	n.s.
Anacyclus radiatus Loisel.	74.3 a	52.7 b	34.2c	29.1	53.4	* *
Anthemis mixta L.	72.6 a	61.5 b	46.5c	15.3	35.9	* *
Blackstonia perfoliata (L.) Huds.	58.2 a	26.4 b	12.3c	54.6	78.9	* *
Centaurea cyanus L.	84.5 a	82.5 a	80.2 a	2.4	5.1	n.s.
Centaurium erythraea Rafn.	52.3 a	34.5 b	19.4c	34.0	62.9	* *
Chrysanthemum coronarium L.	62.7 a	51.7 b	32.7c	17.5	47.8	* *
Chrysanthemum segetum L.	66.2 a	35.4 b	23.0c	46.5	65.3	* *
Consolida regalis Gray	44.3 a	36.6 b	25.4c	17.4	42.6	* *
Lavatera punctata All.	48.9 a	46.2 a	44.5 a	5.5	13.1	n.s.
Legousia speculum-veneris (L.) Chaix	78.1 a	42.9 b	31.4c	45.1	59.8	* *
Myosotis arvensis (L.) Hill	57.3 a	39.6 b	27.9c	30.9	51.3	* *
Nigella damascena L.	88.6 a	86.3 a	84.2 a	11.6	13.4	n.s.
Orlaya grandiflora (L.) Hoffm.	50.4 a	49.5 a	48.8 a	1.8	3.2	n.s.
Papaver rhoeas L.	61.2 a	44.2 b	32.1c	27.8	47.6	* *
Silene conica L.	77.5 a	49.5 b	27.2c	36.1	64.9	* *
Tripleurospermum inodorum (L.) SB	86.3 a	36.4 b	18.4c	57.8	78.7	* *
Vaccaria hispanica (Mill.) Raushert	84.8 a	69.3 b	67.5 b	18.3	19.0	n.s.

1 As decreased emergence percentage to respect to Petri dish germination.

2 Seedling emergence inhibition % as a comparison between the two soil textures.
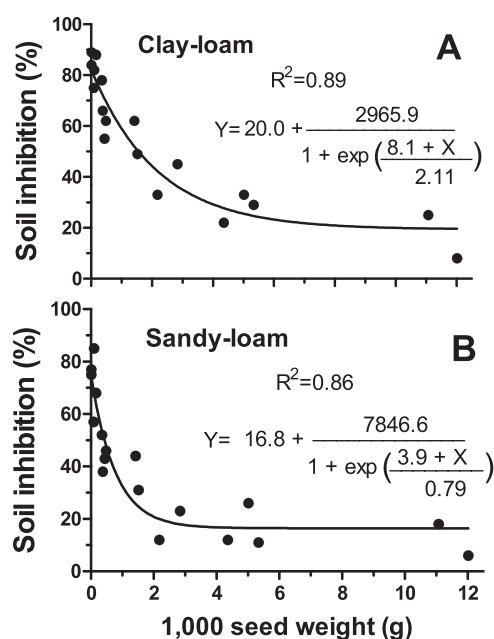

Fig. 3. Boltzmann sigmoidal regression (statistically significant. p < 0.05) between 1000 seed weight and their respective soil inhibition induced by both soil types (A=clay-loam and B=sandy-loam) expressed as emergence percentage to respect to the germination performances detected in Petri dishes.

Fig. 4. Seedlings of the eighteen species of wildflowers during the early growth stage in evident condition of reciprocal interference.

negligible plant mortality which became was much more notable in the sowing in clay-loam soil. In fact all these last mentioned species showed a highly significant mortality (p < 0.01) when comparing the two soil textures. *O. grandiflora* was an exception since it showed a high mortality in clay-loam soil despite the relatively large and heavy seeds.

The flowering period of the eighteen wildflower species (Table 4) lasted from early April (*A. radiatus*, *A. mixta*, *C. coronarium* and *C. segetum*) to the first ten days of July (*L. punctata*). Although each species exhibited a different flowering period, in May all species showed full flowering.
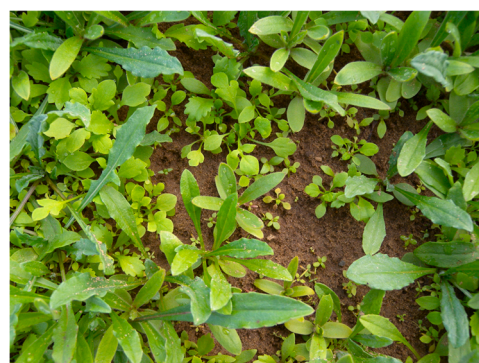
3.3. Pollinator visits

In May, the pollinators that visited the various species of flowers were observed and counted. Pollinator typologies were divided into the following six categories (Fig. 5): bees, solitary bees, dipters (syrphidae and bombyliidae), and lepidoptera.

The total number of visits occurring in the two wildflower communities, based on their bio-agronomic performance in the two soil textures, showed no significant differences (Table 5). In fact, in both cases, the visit rate was nearly 300 pollinators m^{-2} h^{-1}. In contrast, the various categories of pollinators often showed significant differences between the two types of wildflower communities. In particular, while the bees were more often observed (significant p < 0.01) on the wildflowers sown on clay-loam soil (33.2% compared to 25.2% of sandy-loam soil), diptera bombyliidae showed a greater presence in the soil sandy-loam soil (16.2%) than clay-loam soil (7.5%). Further differences, which were also statistically significant albeit to a lesser extent (p < 0.05), were shown by solitary bees and bumblebees. Conversely, diptera syrphidae and lepidoptera showed no significant difference between the two types of wildflower communities.

Table 3
Wildflower density reduction expressed as percentage of plant density during full flowering (detected at half May) to respect to the initial seedling density (detected at half March one month after sowing). Single or double asterisks indicate statistical difference (p < 0.05 or p < 0.01 respectively) according to Duncan's LSD test.

Wildflower species	Density reduction %		Statistical significance
	Sandy-loam	Clay-loam	
Agrostemma githago L.	0	0	n.s.
Anacyclus radiatus Loisel.	2.4	3.7	*
Anthemis mixta L.	2.1	5.2	* *
Blackstonia perfoliata (L.) Huds.	3.4	7.5	* *
Centaurea cyanus L.	0	0	n.s.
Centaurium erythraea Rafn.	4.3	8.1	* *
Chrysanthemum coronarium L.	1.8	3.5	*
Chrysanthemum segetum L.	1.4	2.9	*
Consolida regalis Gray	1.3	3.4	*
Lavatera punctata All.	0	0	n.s.
Legousia speculum-veneris (L.) Chaix	3.3	7.3	* *
Myosotis arvensis (L.) Hill	3.5	8.2	* *
Nigella damascena L.	1.2	3.6	*
Orlaya grandiflora (L.) Hoffm.	1.1	1.7	n.s.
Papaver rhoeas L.	2.2	3.5	*
Silene conica L.	1.9	5.6	* *
Tripleurospermum inodorum (L.) SB	2.0	6.2	* *
Vaccaria hispanica (Mill.) Raushert	1.5	2.6	*
Means	1.8	4.0	*

3.4. Wildflower and pollinator biodiversity

The bio-agronomic performances of the wildflower communities sown in the two soils with different textures showed a different and statistically significant (p < 0.05) level of biodiversity which was revealed by the Shannon indexes (Fig. 6A). The wildflowers grown on sandy-loam soil showed a higher level of biodiversity than those grown on clay-loam soil. Similarly, pollinator biodiversity, expressed by the same Shannon index, was also statistically higher (p < 0.05) in the case of wildflowers grown on sandy-loam soil (Fig. 6B).

4. Discussion

4.1. Seed weight, germination and seedling emergence

Contrary to most crops, whose weight of 1000 seeds is almost always more than at least a few grams, the tested wildflowers we are characterized by very small seeds whose 1000 seed weight is often less than a gram. This appears be a survival strategy, which in natural environments, is capable of producing a large number of seeds that are uninteresting for birds and small rodents to eat (Leishman et al., 2000) by after their dispersal in the surrounding environment.

As expected, the seeds of these tested wildflower species are characterized by a consistent dormancy (Baskin and Baskin, 2004). In fact, even in optimal incubation conditions (Petri dishes), germination exceeded 90% exclusively in *A. githago* while, in the other species, a notable seed dormancy was found. However, germination was lower than 50% only in *C. regalis* and *L. punctata*. This scarce germination appears to arise, in *C. regalis* from the typical embryonic dormancy that is frequent in many Ranunculaceae species (Lee et al., 2018), while in *L. punctata* from physical dormancy as typically occurs in many Malvaceae species (Baskin et al., 2000). However, considering the non-domesticated nature of the selected species, seed dormancy in itself does not appear to be a problem, since almost all of the species showed a germinability over 50%. Unfortunately this level of germination that was detected in optimal conditions often decreases after being sown in the soil. Seed sowing, albeit at a shallow depth of a few millimeters, involves germination-inhibition often consistently. This inhibition seems to be linked to the difficulty in gaseous diffusion within the soil porosity with the consequent difficulty in both i) supplying oxygen and ii) removing the toxic fermentative metabolites that are typically produced during the preliminary stages of germination in a hypoxic environment (Benvenuti, 2003). On the other hand, it should be noted that the lack of high-intensity rainfall during the experimental two-year period (see material and methods) prevented the formation of that surface crust which could have led to "fatal germinations". In other words, the lack of seedling emergence cannot be due to the seedling death during the pre-emergence elongation but was exclusively due to the seed dormancy induction due to the hypoxic atmosphere of the soil matrix.

The soil texture thus plays a crucial role since smaller particles hinder the gas exchange (in-out soil) to a greater extent, highlighting a greater degree of soil-mediated inhibition. A further inhibition of seed germination was related to the seed weight of the tested species. Boltzmann regressions (significant for p < 0.05) showed an inverse

Table 4
Calendar of flowering (the symbol ●=ten-day period) of the tested wildflowers during the experimental season.

Wildflower species	Months				
	March	April	May	June	July
Agrostemma githago L.		●	● ● ●	● ●	
Anacyclus radiatus Loisel.		● ●	● ● ●	●	
Anthemis mixta L.		● ●	● ● ●	● ●	●
Blackstonia perfoliata (L.) Huds.			● ● ●	●	
Centaurea cyanus L.		●	● ● ●		
Centaurium erythraea Rafn.			● ● ●	● ●	●
Chrysanthemum coronarium L.		● ●	● ● ●	● ●	
Chrysanthemum segetum L.		● ●	● ● ●	● ●	
Consolida regalis Gray			● ● ●	● ●	● ●
Lavatera punctata All.			● ● ●	● ● ●	● ● ●
Legousia speculum-veneris (L.) Chaix		●	● ● ●		
Myosotis arvensis (L.) Hill		●	● ● ●		
Nigella damascena L.			● ● ●	●	
Orlaya grandiflora (L.) Hoffm.		●	● ● ●	●	
Papaver rhoeas L.			● ● ●	●	
Silene conica L.			● ● ●	●	
Tripleurospermum inodorum (L.) SB			● ● ●	● ●	●
Vaccaria hispanica (Mill.) Raushert		● ●	● ● ●		

Fig. 5. Some examples of the pollinator categories observed on the flowers of the tested species: 1 = bee (on *N. damascena*). 2 = solitary bee (on *G. segetum*). 3 = bumblebee (on *C. cyanus*). 4 = diptera syrphidae (on *A. mixta*). 5 = diptera bombyliidae (on *L. Speculum-veneris*) and 6 = lepidoptera (on *A. githago*).

Table 5
Flower visits percentages by different pollinator groups (Bees, Solitary bees, Bumblebees, Diptera syrphydae, Diptera bombyliide and Lepidoptera) and total visitation rate observed in wildflower communities sown in soil with different texture. Single or double asterisks indicate statistical difference ($p < 0.05$ or $p < 0.01$ respectively) according to Duncan's LSD test.

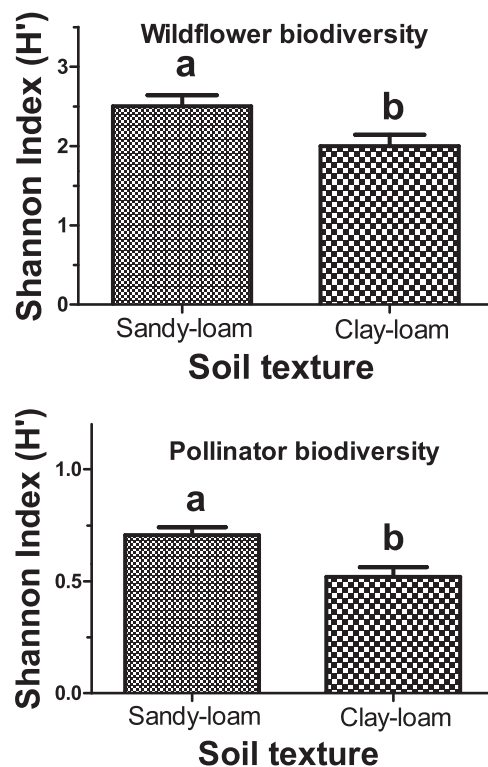| Soil texture	Pollinators groups						Total visitation rate
	Apoidea			Diptera		Lepidoptera	Visits (m^{-2} h^{-1})
	Bees	Solitary bees	Bumblebees	Syrphidae	Bombyliidae		
	(%)						
Sandy-loam	25.2	20.5	10.5	18.4	16.2	12.4	288.4
Clay-loam	33.2	25.4	4.5	18.2	7.5	11.2	292.8
Significance	* *	*	*	n.s.	* *	n.s.	n.s.

Fig. 6. Indexes (Shannon index. H') of wildflower biodiversity (top) detected in the two different soil texture (during the full flowering phenological stage) and the respective pollinator biodiversity (bottom) detected during the May. Means with different letters indicate significant differences (P < 0.05) according to the Duncan's Multiple Range Test. Vertical bars indicate the ± standard error of the means.

relationship between the 1000 seed weight of the eighteen wildflower species and the corresponding inhibition of seed germination and seedling emergence, thus confirming findings in other wild species (Benvenuti and Mazzoncini, 2021), especially in clay-textured soils (Benvenuti and Mazzoncini, 2018). On the other hand, small seeds, at least in wild herbaceous species, enter secondary dormancy easily if incorporated into a soil matrix that is rich in clay particles that hinder gaseous diffusion during the early stage of germination. This is because the lack of gaseous diffusion increases the level of hypoxia, thus inducing seed dormancy (Benvenuti and Macchia, 1995).

From an agronomic point of view, very small seeds (i.e. *B. perfoliata, C. erythraea, A. radiatus, L. speculum-veneris* and *T. inodorum*) are not suitable as agro-ecological restoration strategies, especially for sowing on silty and/or clayey soils. Conversely, wildflower species that have larger and heavier seeds (i.e. *A. githago, O. grandiflora, V. hispanica* and *C. cyanus*) showed the best agronomic performance not only in the less inhibiting sandy-loam texture soil but also in the "more inhibiting" clay-loam soil.

4.2. Dynamics of wildflower density and flowering

As expected, strong interference affected the emerged seedlings of the eighteen species (Fig. 4) both due to their competitive (Bybee-Finley et al., 2022) and allelopathic (Hoffman et al., 1996) nature. This led to a non-negligible seedling mortality as typically occurs in small-seeded crops sown with a high-seed density (Hall et al., 2004). However, this seedling mortality was also due to the soil texture confirming previous observations (Bretzel et al., 2009). In particular, the clay-loam soil resulted in a higher mortality especially for species with very small seeds (i.e. *C. erythrea, L. speculum-veneris* and *T. inodorum*).

On the other hand, a direct relationship between the seed weight and

survival of the relative seedlings has already been found in other wild species (Moles, Westoby, 2004). Consequently, the small-seeded species, whose emergence was already more inhibited by the clay-loam texture, suffered greater competitive interference with the more vigorous species (i.e. *A. githago* and *C. cyanus*) characterized by larger seeds (Geritz et al., 1999). In practice, the scarce energy reserves of small seeds determined scarce competitiveness in the related seedlings. Their increased mortality thus appears to be due to the light scarcity, as typically occurs under the dense canopy of the more vigorous seedlings derived from the larger seed species (Leishman and Westoby, 1994).

However, the plant mortality of the wildflower species with small seeds was decidedly lower in the sandy-loam soil, probably due to the greater uniformity in their emergence dynamics. This highlights their usability for wildflower strip sowing, but almost exclusively in the case of sandy soils.

Despite the different mortality of the two wildflower communities, the relative flowering dynamics were not influenced by the soil texture. In both soil textures, May was the period of full flowering for all the species. In terms of the food availability (pollen and nectar) provided by both wildflower communities, such botanical taxa appear to be fully suited to pollinator survival during the late spring periods. Basically, a wide range of flowering dynamics was detected ensuring food available for pollinators as early as April by the earliest species (i.e. *A. radiates, A. mixta, C. coronarium* and *C. segetum*) until the end of June by the later species (i.e. *B. perfoliata, C. regalis, L. punctata* and *T. inodorum*). The latest flowering species such as *C. regalis* and *L. punctata* (flowering until June) appeared to be the ideal species for prolonging the food availability until the dry periods, which are frequently low in pollen and nectar availability as typically occurs in the Mediterranean environment (Benelli et al., 2014).

4.3. Pollinator visits

Despite the different emergence rate and plant mortality of the two wildflower communities, the non-limiting quantity of pollen and nectars of both blooms resulted in a similar visitation rate by pollinators. However, their different botanical complexity (with fewer small-seeded species sown in the clay-textured soil) modified the pollinator biodiversity. The higher bee visit rate, detected in the clay-loam textured soil, seems to be related to the higher dominance of fewer species (overall larger-seed species since decreased the small-seeds tend to decrease). This could be explained by the fact that bees are typically attracted by the dominance of a few species. This behaviour, referred to as "constancy" (Grüter et al., 2011), is the tendency to visit daily only one species (the most abundant) and not the flowers of other species. In contrast, the higher rate of visits by Diptera bombyliidae, of wildflowers sown in sandy-loam soil, appears to be due to the higher presence of flowers characterized not only by small seeds but also by small flowers (i.e. *B. perfoliata, C. erythrea, L. speculum-veneris, M. arvensis, T. inodorum*. In fact, these small pollinators, although not exclusively specialized for certain floristic *taxa*, are frequently observed on very small flowers (Kastinger and Weber, 2001), thanks to their typical very elongated mouthparts that are capable of sucking nectar (Szucsich and Krenn, 2002). Similarly, the different visit rate of bumblebees and solitary bees can also be explained by the respective degree of preferences for the available flower typologies. Finally, the rate of visits of diptera syrphydae and lepidoptera were similar in the two wildflower communities, suggesting a poor specialization, at least for the wildflowers tested, in the choice of flowers to forage.

4.4. Wildflower and pollinator biodiversity

Although the wildflower seed mixes were planned taking into account the germinability of each species (amount of seeds inversely proportional to the relative dormancy), their germination and seedling emergence were strongly influenced by the soil texture. The fact that

clay-loam hindered seedling emergence and plant survival for small-seed species led to a reduction in biodiversity compared to sowing the same seed mix in sandy-loam soil. In practice, sandy soils appear more suitable for expressing the expected biodiversity of the selected wild-flower seed mixture. However, to achieve a high biodiversity in wild-flower strips sown on clayey soils, a large number of species characterized by relatively larger seeds is advisable. This then leads to a greater floristic balance thanks to the lower inhibition of their seed germination and seedling emergence.

It is important to underline that the floristic biodiversity was proportional to the pollinator biodiversity, confirming our previous experiments (Benvenuti, 2022) aimed at restoring the biological complexity of the agroecosystem. In fact the higher Shannon index of the wildflower mix sown in sandy-loam soil corresponded to the higher Shannon index of the relative pollinators. This confirms that the establishment success of wildflower strips is strongly dependent on the choice of species (Scheper et al., 2021) that are known to achieve optimal performance in the particular ecological conditions where the wild-flower strips are sown.

5. Conclusions

Soil texture is an important aspect for planning wildflower strips. In fact, the achievement of the expected biodiversity derives from optimizing the compatibility between soil texture and wildflower seed weight. Basically, the choice of plant species to include in the wildflower mix should be based on their characteristics in terms of weight and size. In particular, clay-rich soils suit species with heavier seeds (approximately 1000 seeds weighing more than 2 g) in order to prevent a decline in the predicted biodiversity due to the germination constraints of the small-seed wildflower species. This knowledge could be used as a tool for biodiversity restoration in a wide range of ecological conditions, which will help safeguard the ecological health and biodiversity of degraded agroecosystems.

CRediT authorship contribution statement

Stefano Benvenuti: Conceptualization, Investigation, Data curation, Writing – review & editing.

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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