Herbaceous vegetation enhancement increases biodiversity in a wine-producing vineyard in Israel, promoting shifts in agricultural practices in other vineyards

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SUMMARY

We tested the effects of herbaceous vegetation enhancement on the abundance and richness of plants and arthropods in a wine-producing vineyard in Israel. We compared the abundance and species richness of plants and arthropods between a plot seeded with local annual plants and an unseeded plot. We also compared soil content and grape quality parameters in seeded versus unseeded plots in the vineyard. Seeding increased plant cover and plant species richness in the spring, but reduced plant cover and did not affect species richness in summer. Arthropods, and especially parasitoids and generalist predators, were more abundant and diverse in the seeded than in the unseeded plots in spring, both on the herbaceous vegetation and on the vine foliage. Arthropods were more abundant in the herbaceous vegetation than on the vine foliage in spring, but not in summer. The soil in seeded plots was richer in ammonium nitrogen and organic matter, while the grapes were smaller and sweeter. Our findings showing a general increase in biodiversity, combined with additional considerations, led the managers of the vineyard to implement these vegetation enhancement practices in 85% of their vineyards.

BACKGROUND

Growing concerns regarding the negative effects and economic consequences of intensive chemical use in agriculture on biodiversity, soil health, ground water reservoirs and human health (Wilson & Tisdell 2001, Foley *et. al*. 2005, Pimentel 2009) have motivated exploration of alternative agricultural practices. Actions for enhancing agricultural sustainability are varied, and include supporting and maintaining low-intensity agricultural methods, increasing the proportion of semi-natural and natural habitats in the farmed landscape, implementing food labelling schemes for environmentally friendly farming and promoting wild vegetation around and within agricultural fields (Dicks *et al*. 2013).

Diversification of vegetation in agroecosystems was found to promote ecosystem services such as recycling of nutrients, regulation of microclimate and local hydrological processes, detoxification of noxious chemicals and reduction of soil erosion (Altieri 1999, Dicks *et al*. 2013). Naturally, the addition of plant resources supports biodiversity in general and arthropods in particular (Dicks *et al*. 2013). Moreover, in the context of pest control, these resources can potentially sustain predators and parasites of agricultural pests.

Integrated pest management implements biological, chemical, physical and agrotechnical approaches for pest control and aims at reducing its economic, health and environmental costs (Kogan & Bajwa 1999). Within this field, conservation biological control espouses on-site manipulations to support the natural enemies of agricultural pests, by encouraging herbaceous vegetation growth either around or within the plots, or both. Successful conservation biological control may allow growers to reduce or even replace the use of herbicides and pesticides. These actions can potentially increase the biodiversity of both flora and fauna, and can benefit agricultural and financial measures in the short and long term.

The abundance and diversity of natural enemies usually correlates positively with the abundance and diversity of natural herbaceous vegetation in and around agricultural fields (Langellotto & Denno 2004, Letourneau *et al*. 2011). In contrast, the effects of vegetation conservation on pest population dynamics and abundance are apparently more complex, and vary between increases, indifference and decreases (Letourneau *et al*. 2011, Winqvist *et al*. 2011, Chisholm *et al*. 2014). Therefore, recommendations for sustainable agricultural practices should be based on crop- and site-specific studies, and should consider beneficial organisms and total arthropods separately.

Wine-grape vineyards in Israel are suitable candidates for shifting towards more sustainable agricultural interfaces due to their relatively small field sizes, low chemical and water inputs, and the wineries' demands for quality rather than quantity of grapes. Incentives for environmental branding (see for example SWSA 2016), and the potential for increasing biodiversity in ecologically sensitive sites (where many vineyards in Israel are located) provide additional motivation for implementing environmentally friendly measures.

To promote large-scale changes it is probably necessary to join forces between parties of mutual interests. Here we report on a joint study involving wine producers, growers, a nature conservation NGO and scientists, which was funded by the Tabor winery (www.twc.co.il) and the Nekudat Hen Fund for sustainable agriculture (http://www.nekudat-hen.org.il/en). The study took place in a commercial wine vineyard as a test case, with the aim of shifting agricultural practices from

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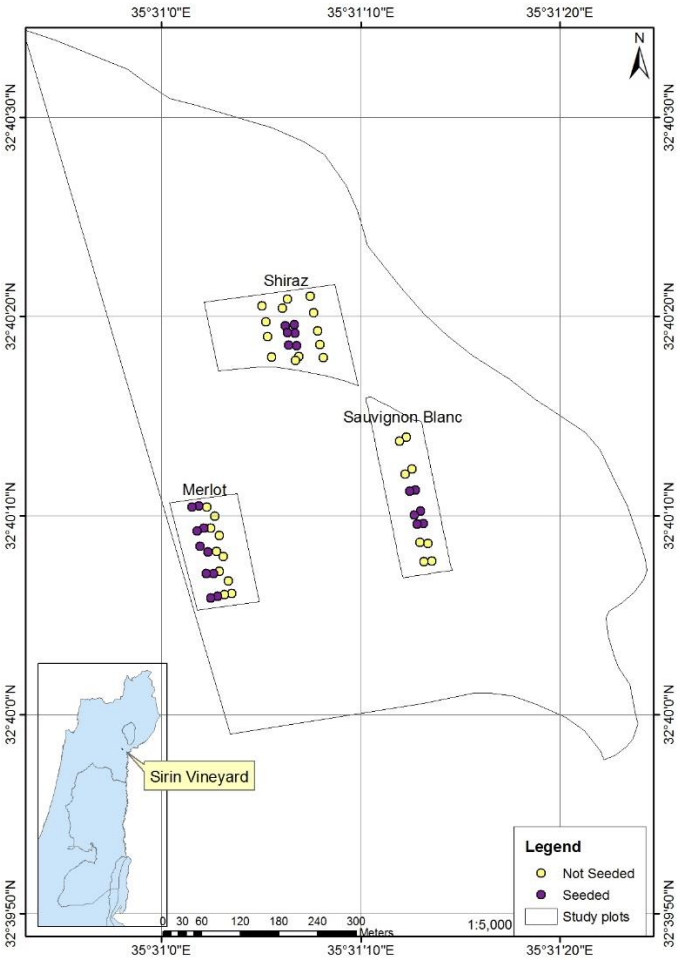

Figure 1. Map of the study area, experimental plots and sampling points of herbaceous vegetation and arthropods.

conventional to more sustainable in other wine-producing vineyards across the country. We tested how enhancement of native herbaceous vegetation affects the in-field richness of plants and of herbivorous, predatory and parasitoid arthropods. In addition, we tested the effect of vegetation enhancement on some soil properties and grape parameters, two significant agricultural factors that can affect crop quality. Our study provides a first evaluation of the agro-ecological consequences of management practices involving herbaceous vegetation in Israeli vineyards.

ACTION

The study was conducted in a 37 ha vineyard, located at the Sirin Highland, Israel (32°39'2.34"N, 35°31'10.19"E). The vineyard is planted on basalt-derived reddish brown Grumusol soils and is managed by the Tabor winery. It comprises multiple lots, each planted with a different grape cultivar, including Sauvignon Blanc, Shiraz, Merlot and Petite Sirah (Figure 1). The vineyard was under conventional management until our study was initiated, namely was maintained as a "clean vineyard" with bare soil and regular applications of herbicides and pesticides.

In the conventional practice of vineyards in Israel it is common to prevent any growth of weeds through applications of pre-germination herbicides in fall and spring, which target mainly local winter- and summer-sprouting annuals. In addition to the impact on non-crop plants, this practice can also affect the abundance, composition and distribution of herbivorous arthropods and their natural enemies. It was therefore important to document the abundance of arthropods, particularly natural enemies, and also where they were found in vineyards, as this helps assess what aspects of the habitat are important for them.

Table 1. Herbaceous plants seeded in experimental plots.

Anemone coronaria; *Anthemis palestina*; *Bromus madritensis*; *Chrozophora tinctoria*; *Chrysanthemum segetum*; *Echium judaeum*; *Erodium gruinum*; *Erucaria hispanica*; *Heliotropium spp.*; *Hordeum glaucum*; *Lagurus ovatus*; *Lavatera cretica*; *Linum pubescens*; *Papaver umbonatum*; *Ranunculus asiaticus*; *Ridolfia segetum*; *Scabiosa prolifera*; *Schedonorus arundinaceus*; *Senecio leucanthemifolius*; *Trifolium campestre*; *Trifolium purpureum*; *Trifolium repens*; *Vicia villosa*

In this study, we evaluated how seeding local herbaceous plants between the vine rows affected (1) the abundance and species richness of herbaceous plants, (2) the abundance and richness of all arthropods on vegetation and on vine foliage, (3) the abundance of potential arthropod natural enemies (parasitoids and the most abundant predators - spiders and antlions), on vegetation and vine foliage, (4) soil nutrient properties, and (5) grape quality indices. Herbaceous vegetation treatment plots were seeded in 2013-2014. Soil nutrient properties and grape quality indices were sampled during 2014, while herbaceous vegetation and arthropods were surveyed and sampled in 2015.

Seeding: One kilogram of seeds of low-growing local flowering annuals (see Table 1 for species) were dispersed in two 0.1 ha rectangular seeded plots during the fall seasons of 2013 and 2014. One seeded plot was located in the Shiraz area, and one in the Sauvignon Blanc area, and each spread over several vine rows. During fall 2014 we seeded a further 0.1 ha plot within the Merlot area (Figures 1, 2). Seeded plots were not sprayed with herbicides. Unseeded plots within the same area of grape cultivar were considered as controls and were treated with herbicides. During 2014 the use of herbicides was discontinued between the vine rows (but continued within the vine rows) in all cultivar areas except for the unseeded parts of the Merlot area, where spraying was continued. Thus, the Shiraz and Sauvignon Blanc areas provided a comparison

Figure 2. A vineyard plot seeded with local annuals and no herbicide treatment (left) and an unseeded plot treated with herbicides (right), during springtime (pre trimming).

between seeded and spontaneously developing herbaceous vegetation, while the Merlot area provided a comparison between seeding and herbicide control of weeds. This meant that there was only a single seeding treatment and herbicide control site for the comparison of vegetation and arthropods, and the results could therefore be site- or cultivar-specific. The annual vegetation seeded in December 2013 was mechanically trimmed during May 2014 due to an unexpected drought event that led to competition for water with the vines. The annuals seeded in 2014 were trimmed in June 2015, after they had completed their life-cycle, dispersed seeds and dried out. The trimmed plant material and the dry biomass of the annuals below trimming height, as well as their below-ground biomass, remained in the plots.

Vegetation sampling: We surveyed the herbaceous vegetation during May 2015 (before trimming) and July 2015 (after trimming) for percentage cover and species richness. We sampled 20 rectangles of 2 x 1 m in the Merlot cultivar area (10 between rows in the seeded plot and 10 between rows in the control herbicide unseeded plot).

Arthropod sampling: We used a Vortis insect suction sampler to collect arthropods from herbaceous vegetation between the vine rows and from the vine foliage in the Merlot area (20 samples from seeded rows and 20 samples from unseeded control rows per collection date). Sampling duration was 15 seconds. All samples were preserved in 75% ethanol at 4 ºC. Most arthropods were sorted to the order level. However, some taxa, which were of special interest as potential pests or natural enemies (such as parasitoid wasps), were classified to lower levels (see Table 2). Taxonomic groups with fewer than 10 specimens across all samples were omitted from further analyses.

Arthropods were sampled once a month, between April and July 2015. The April and May samples were collected before the herbaceous vegetation was trimmed, while the June and July samples were collected post-trimming. April is mid-spring in Israel (average temperatures of 20-25 °C and 40-50% humidity at sampling) and the beginning of vine blooming. July is high summer (average temperatures rising to 35-40 °C and 55-65% humidity). Samples were taken between 09:00 and 12:00 h.

Soil sampling: Soil samples from depths of 0-5 and 5-15 cm were randomly collected from seeded and unseeded control plots in the Shiraz and Sauvignon Blanc cultivar areas during May 2014. In the Shiraz area, six samples were collected from seeded rows and 13 from unseeded control rows. In the Sauvignon Blanc area, six samples were collected from seeded rows and eight from unseeded control rows. Soil samples were analyzed for mineral nitrogen (nitrate ($N\text{-}NO_3$) and ammonium ($N\text{-}NH_3$) by extraction with 2M KCl (Keeney & Nelson 1987).

Table 2. Taxonomic levels of arthropod sorting.

Taxonomic level	Taxon
Subclass	Acari, Collembola
Order	Coleoptera, Psocoptera, Neuroptera, Orthoptera, Thysanoptera, Lepidoptera, Araneae, Hymenoptera (parasitoid wasps).
Suborder	Heteroptera, Brachycera, Nematocera, Apocrita (Parasitica)
Family	Formicidae, Aphidoidea, Aleyrodidae

Available phosphorus ($P\text{-}PO_4$) was determined using the Olsen method (Olsen & Sommers 1982) and soluble potassium (K) was removed by a single extraction in 1 M HNO_3. Soil organic matter (SOM) content was measured by weight loss on ignition to 400 ºC (Ben-Dor & Banin 1989).

Grape quality indices: The winery routinely monitors fruit development to optimize their harvesting date. In August 2014, eight grape bunches from each of the soil sampling points in the Shiraz and Sauvignon Blanc cultivar areas (see above) were used to compare seeded and unseeded control plots. We weighed the bunches and extracted the grape content. We determined the grapes' sugar levels using a refractometer (measuring total soluble solids, 98% of which are sugars), pH using a liquid pH meter and total acidity levels using titration.

Data analysis

Vegetation: We used paired-sample t-tests to compare the percentage cover of herbaceous vegetation and the number of plant species per sampling rectangle between seeded and unseeded control rows in the Merlot cultivar area in March (spring) and July (summer) 2015, which corresponded with pre- and post- trimming of the seeded rows.

Arthropods*:* We averaged the numbers of arthropods per sample for April-May and for June-July to get a pre- and post-trimming mean abundance in the Merlot cultivar area. We then multiplied the average values by the proportion of the corresponding point's vegetation percentage cover, to calculate overall relative abundance. Tests were run separately for pre-trimming (spring) and post-trimming (summer).

We compared total arthropod abundance, abundance of potential natural enemies, and arthropod taxon richness (with classification into taxa as specified in Table 2) in the Merlot cultivar area between (a) samples in seeded and unseeded control rows, (b) samples from herbaceous vegetation vs. vine foliage and (c) pre- and post-trimming samples. Whenever we performed two tests on the same data, we used $p = 0.025$ as our significance threshold. The most common potential natural enemies (NE) included in analysis were parasitoid wasps, spiders and antlions. As the values were not distributed normally we used Wilcoxon signed-rank tests for these comparisons.

Soil: We compared the concentrations of the major soil nutrients (NPK) and soil organic matter between soil samples from seeded and unseeded control rows in the Shiraz and Sauvignon Blanc cultivar areas. The influence of grape cultivar, seeding, depth of soil sampling and their interactions on each of the soil parameters were examined using general linear models.

Grape quality: We compared grape quality indices (bunch weight, total acidity, sugar content (Brix index) and pH) between samples from seeded and unseeded control rows in the Sauvignon Blanc cultivar area in 2014, using t-tests.

CONSEQUENCES

Herbaceous vegetation in the Merlot cultivar area: Vegetation cover in the Merlot area was significantly higher in the seeded plot than in the unseeded control plot during pre-trimming ($t = 9.81$, d.f. = 9, $p < 0.001$), but higher in the

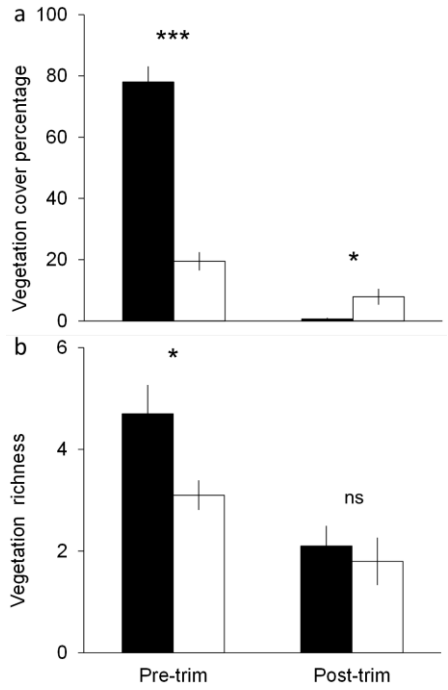

Figure 3. (a) Herbaceous vegetation cover and (b) species richness in treatment and control rows in the Merlot cultivar area during March (pre-trimming) and July (post-trimming) 2015. Filled columns represent seeded plot, unfilled columns represent unseeded control plots. P-values are denoted by * < 0.05; **<0.01; ***<0.001.

unseeded control plot compared with the seeded plot post-trimming (t = 2.95, d.f.= 9, p = 0.016; Figure 3a). Herbaceous vegetation richness (measured as the number of species) was significantly higher in the seeded plot than in the unseeded control plot in the pre-trimming period (t = -2.77, d.f.= 9, p = 0.022; Figure 3b). There was no significant difference between the seeded plot and the unseeded control plot in species richness post-trimming (t = 0.64, d.f .= 9, p = 0.54; Figure 3b).

Effects of seeding on arthropods in the Merlot cultivar area: Total arthropod abundance was significantly higher in the seeded plot than in the unseeded control plot pre-trimming (p < 0.001). It was slightly, but not significantly, higher in the unseeded control plot compared with the seeded plot post-trimming (p = 0.072, Figure 4a). The number of arthropod taxa was significantly higher in the seeded plot than in the unseeded control plot pre-trimming (p <0.001) and significantly higher in the unseeded control plot compared with the seeded plot post-trimming (p < 0.001, Figure 4b). NE abundance was also significantly higher in the seeded plot than in the unseeded control plot pre-trimming (p = 0.002), and higher in the

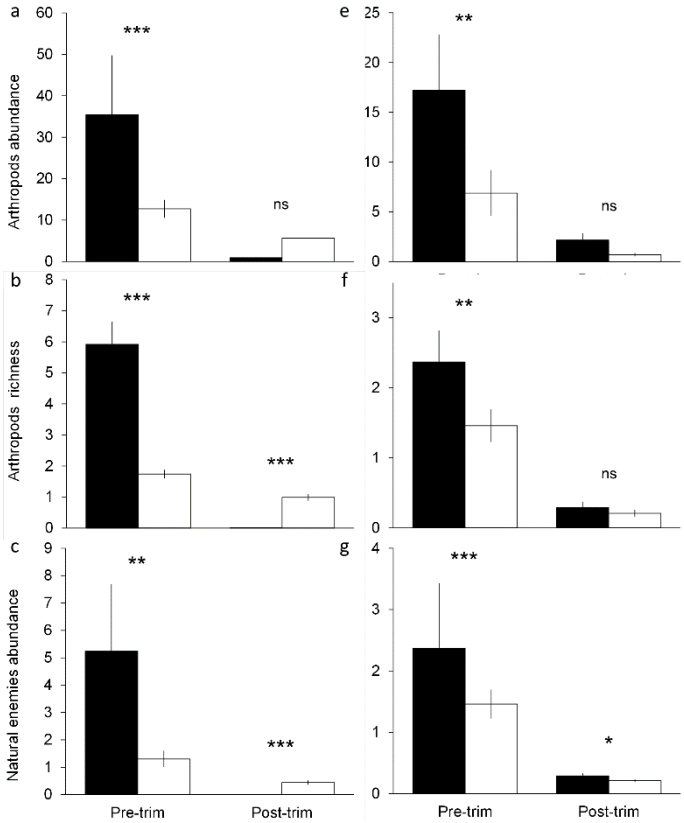

Figure 4. (a) Arthropod abundance, (b) arthropod richness and (c) natural enemy abundance in seeded plot (filled columns) versus unseeded control plot (unfilled columns) in the Merlot cultivar area during April-March (pre-trimming) and June-July (post-trimming). Ground vegetation and vine foliage samples were combined. (d) Arthropod abundance, (e) arthropod richness and (f) natural enemy abundance in ground vegetation (filled columns) versus vine foliage (unfilled columns) in the Merlot cultivar area during April-March (pre-trimming) and June-July (post-trimming). Seeded and unseeded control samples combined. P-values are denoted by * < 0.05; ** < 0.01; *** < 0.001.

unseeded control plot than in the seeded plot post-trimming (p < 0.001, Figure 4c). Seeding did not affect the abundance of NE within the vine foliage (p = 0.26).

Arthropods in herbaceous vegetation versus vine foliage in the Merlot cultivar area: Arthropod abundance and the number of taxa were significantly higher in the herbaceous vegetation than in the vine foliage during pre-trimming, but not post- trimming (abundance: p = 0.004, p = 0.067 respectively, Figure 4d; number of taxa: p = 0.021, p = 0.32 respectively,

Table 3. Effects of cultivar plots (Shiraz, Sauvignon Blanc), seeding (seeded, unseeded) and depth of bore hole (5 cm, 15cm) on soil properties (soil organic matter (SOM) and major nutrients), derived from general linear models. Values in bold denote significant effects.

	SOM		**N-NO$_3$**		**N-NH$_4$**		**P-Olsen**		**K-CaCl**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Corrected model	**5.35**	**>0.001**	1.10	0.38	**2.34**	**0.04**	0.78	0.61	1.64	0.15
Plot	**26.10**	**>0.001**	2.96	0.09	**8.40**	**0.01**	2.04	0.16	0.82	0.37
Seeding	**5.65**	**0.02**	0.00	0.98	3.65	0.06	0.33	0.57	0.95	0.34
Depth	0.10	0.75	1.58	0.21	0.57	0.46	0.07	0.79	5.42	0.02
Plot*Seeding	0.12	0.74	2.72	0.11	0.05	0.82	1.98	0.17	0.69	0.41
Plot*Depth	0.33	0.57	0.01	0.93	0.05	0.83	0.08	0.77	0.04	0.84
Seeding * Depth	0.02	0.89	0.07	0.80	0.21	0.65	0.01	0.94	0.18	0.67

Table 4. Soil properties values (mean ± s.d.) in samples from Shiraz and Sauvignon Blanc cultivar plots, and seeded and unseeded areas within each plot.

	Shiraz		Sauvignon	
Indices	**Seeded plot**	**Unseeded plot**	**Seeded plot**	**Unseeded plot**
SOM (%)	4.23 ± 0.74	3.68 ± 0.91	3.12 ± 0.56	2.17 ± 0.71
$N\text{-}NO_3$ (g/kg)	3.48 ± 3.17	4.84 ± 3.45	3.42 ± 2.94	2.02 ± 2.54
$N\text{-}NH_4$ (g/kg)	9.42 ± 7.02	6.89 ± 5.42	5.72 ± 2.43	3.43 ± 2.14
P-Olsen (g/kg)	22.8 ± 6.10	24.9 ± 8.61	22.7 ± 12.7	17.7 ± 5.25
K-CaCl (g/kg)	6.83 ± 1.84	6.76 ± 1.65	7.58 ± 1.31	6.80 ± 1.69

Figure 4e). NE abundance was significantly higher in herbaceous vegetation compared with vine foliage both pre- and post- trimming ($p < 0.001$, $p = 0.045$ respectively, Figure 4f).

Soil properties: General linear models of the different soil properties examined indicated significant differences between plots only for ammonium nitrogen ($N\text{-}NH_4$) ($F_{7,57} = 2.34$, $p = 0.038$) and organic matter content (SOM) ($F_{7,57} = 5.35$, $p < 0.001$). No significant differences were found for nitrate ($N\text{-}NO_3$), potassium (K) and phosphorus (P) (Table 3).

The analysis explained a low percentage of the variance in $N\text{-}NH_4$ (adjusted $R^2 = 0.14$). $N\text{-}NH_4$ concentrations were higher in the Shiraz cultivar area compared with the Sauvignon cultivar area (Table 4; $p = 0.006$, $Eta^2 = 0.144$) (Eta^2 shows the effects size for each factor in an ANOVA analysis; a higher Eta^2 represents a greater the effect on the dependent variable (Sokal & Rohlf 1995). $N\text{-}NH_4$ levels were 36% and 54% higher in samples from seeded areas than in samples from unseeded areas in the Shiraz and Sauvignon plots, respectively (Table 4), but the influence of seeding was not significant ($p = 0.062$; $Eta^2 = 0.068$). Depth and interactions between the main effects were not significant.

The general linear models explained a moderate fraction of the variance in the percent of SOM in the samples (adjusted $R^2 = 0.35$). SOM was 35% higher in the Shiraz compared with the Sauvignon cultivar areas ($p < 0.0001$, $Eta^2 = 0.343$, Table 4). SOM was 15% higher in samples from seeded areas in both areas ($p = 0.021$, $Eta^2 = 0.102$). Depth and interactions between the main effects were not significant.

Grape quality: Grape bunch mass and total acidity were significantly lower in samples from seeded plots compared with samples from unseeded control plots in 2014. In contrast, sugar levels and pH levels were significantly higher in the seeded plots' samples compared with samples from the unseeded control plots (Table 5).

Table 5. Average (s.d.) quality indices for grape bunches from seeded and unseeded rows during August 2014 (two-sample t-test assuming equal variances). Twelve samples were collected from seeded rows, 21 samples from unseeded rows. Each sample comprised eight bunches of grapes.

Indices	**Seeded samples**	**Unseeded samples**	**t**	**p**
Bunch mass (g)	143.5 (6.26)	210.8 (16.7)	-4.32	0.005
Total acidity (g/L)	4.48 (0.1)	4.98 (0.28)	-3.43	0.014
Sugar conc. (°Bx)	24.03 (0.74)	21.98 (0.39)	4.93	0.003
pH	3.58 (0.04)	3.48 (0.03)	4.2	0.006

DISCUSSION

Our results suggest that enhancement of herbaceous vegetation between the vine rows increased in-field abundance of plants and natural enemies, and species richness of both plants and arthropods in general, especially before trimming of the ground vegetation (Figures 3 and 4). These results agree with several previous studies (Thomson & Hoffman 2009, Letourneau *et al*. 2011, Winqvist *et al.* 2011, Dicks *et al.* 2013). However, the literature shows inconsistency of vegetation-arthropods dynamics in vineyards, suggesting that site-specific studies are highly important to determine local strategies (Chisholm *et al*. 2014, Letourneau *et al*. 2011, Winqvist *et al*. 2011). Therefore the current results are of importance, despite the fact that they come from only a single cultivar area in a single vineyard. It is worth noting that arthropod abundance and taxon richness were greater in the herbaceous vegetation than on the vines (Figure 4d-f) even when vegetation cover was low, implying further the importance of non-crop vegetation (compared with vine foliage) as suitable habitat for conserving arthropod diversity.

Interestingly, differences between the seeded and the unseeded control plots exhibited opposite processes. The seeded plot supported higher vegetation cover and general species richness during spring and before trimming, whereas the unseeded control plot was richer in plants and arthropods during summer and after trimming (Figures 3a-b, 4). This phenomenon is probably due to the hay of the winter vegetation, left in the seeded plot after trimming, which suppressed growth of summer vegetation in the seeded plot (Kobayashi *et al.* 2004). Some of the more common local summer-growing plants are exotic or locally invasive species, most notably from the genus *Amaranthus*. The gradual displacement of these species by native and non-invasive species is desirable and was one of the objectives of this study. It is therefore recommended to separate these two types of species in future studies, in order to understand if this is driving the observed result. The decline in vegetation cover between vine rows during the summer also stresses the contribution of uncultivated vegetation islands (Shapira *et al.* unpublished data), which can support high abundance of arthropods year-round and compensate for periods with low vegetation cover between vine rows.

Ground vegetation enhancement in two other cultivar areas increased ammonium concentration and organic matter percentage in the soil after only one season. This increase suggested that ground vegetation remains may act as natural fertilizers to the soil, and hence reduce the need for chemical fertilization, saving time and money for the farmer. Reduction in chemical fertilizers can potentially also reduce the risks of soil salination (Savci 2012) and groundwater contamination in pollution-susceptible areas.

In 2014 we found significant differences in the indices of grape quality between seeded and unseeded samples (Table 5). Bunch mass and sugar index are controlled by the amount of water available to the vines (Zsófi *et al*. 2011). Since this was the first year of the study the farmer did not adjust water quantities to the vines. Therefore vines growth in the seeded plots was retarded relative to other vines. This is probably the reason for the lower weight of the grapes and the higher sugar index values in the seeded plots.

Grape harvest dates are adjusted to achieve berries with optimal sugar levels for wine production. Our findings from 2014 suggest that grapes grown in the presence of herbaceous vegetation possibly ripen faster. Farmers and wineries would

like to control grapes' ripening rate, and this can be achieved by adjusting the vineyards' irrigation schedule to the presence of weeds. The faster maturation of grapes in the seeded plots strengthens the evidence for competition between herbaceous vegetation and vines over water resources (Ruiz-Colmenero *et al*. 2011). Some water stress is a vital component for achieving desirable berry quality parameters (Zsófi *et al*. 2011). Therefore, the use of herbaceous vegetation between the vine rows could potentially give better control over the amount of stress the growers can impose on the vines.

The joint effort reported here has already promoted changes. Since the end of the study, the Sirin vineyard has gone through a total shift in agricultural interface from traditional to sustainable. Herbaceous vegetation is currently trimmed all over the vineyard, whereas spraying is practised only along the narrow belt of the planting line of the vines. In the near future even this practice will cease, with the purchase of a special trimmer that can trim between the vine stems. Additional rocky hedges were built between the vineyard's lots and native broadleaf trees were planted around the edges of the vineyard. Up to date, 85% of the Tabor winery vineyards across Israel have adopted similar practices.

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