Efficiency of two methods of sampling used to assess the abundance and species diversity of adult Syrphidae (Diptera) in mountainous meadows in the Austrian and Swiss Alps

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Abstract. The outcome of assessments of the biodiversity of a taxonomic group often depend on the sampling method. The choice of an adequate method is especially important for biomonitoring purposes. In this study, the effectiveness of two methods of sampling syrphids (Diptera: Syrphidae) is compared: observation plot method vs. line transect, both sampled by sweep netting. Altogether, 18 meadows were selected in three mountain regions in the Austrian and Swiss Alps. We recorded a significantly higher abundance and richness of syrphids using the observation plot method than the line transect method in 2015. Comparing data for one region recorded in 2015 and 2016, similar results were obtained. Syrphid species assemblages were affected by sampling method in both years. More syrphid species and individuals were recorded using the observation plot method, which makes it more suitable for studies aiming at comparing differences in the numbers of adult syrphids in different grassland habitats.

INTRODUCTION

Biodiversity assessments for prioritizing conservation targets often rely on species inventories (Pimm et al., 1995). In addition to biodiversity assessments, species inventories also facilitate environmental monitoring, i.e. by comparing results of standardized sampling methods (Kohlmann, 2011). In selecting the taxa to be sampled, priority must be given to a group that has an important role in the structure and function of the target ecosystem (García-López et al., 2011). Syrphids (hoverflies) are used frequently to assess biodiversity (Ricarte & Marcos-García, 2008; Petanidou et al., 2011) and functional diversity (Schweiger et al., 2007) of a variety of ecosystems. They are important agents of biological control and pollination (Haenke et al., 2009; Petanidou et al., 2011) and are also used as bioindicators (Burgio & Sommaggio, 2007).

The effectiveness of sampling methods has a strong effect on the quantification of adult syrphid communities (Namaghi & Husseini, 2009). Depending on the aim of the study, several methods can be used for sampling adult syrphids, including yellow pan traps, Malaise traps and hand nets. Hickman et al. (2001) report that only hungry syrphids flew around yellow pan traps. If the syrphids are not

hungry there is no reason for them to be attracted by yellow pan traps (Namaghi & Husseini, 2009). Similarly, Cane et al. (2000) cautions that this type of trap may not accurately reflect the diversity of pollinators and is the least effective method of sampling adult syrphids. The line transect (Sommaggio, 1999; Haenke et al., 2009) and the observation plot methods (Frank, 1999), which are both sampled using sweep nets, are used to record adult syrphid abundance and richness.

Therefore, we tested the effectiveness of the line transect and the observation plot methods in alpine meadows in Austria and Switzerland, which are known to harbour a large diversity of syrphids (Hussain et al., 2017). These two methods were used because they are suitable for use in alpine meadows. Moreover, these methods were studied because they are regularly used but usually separately (Frank, 1999; Sobota & Twardowski, 2004; Marcos-García et al., 2012; Mudri-Stojnić et al., 2012; Power et al., 2016; Walcher et al., 2017). The aim of the present work was to determine which of these two sampling methods recorded the most species and individuals of adult syrphids. Moreover, we investigated whether the species assemblages re-
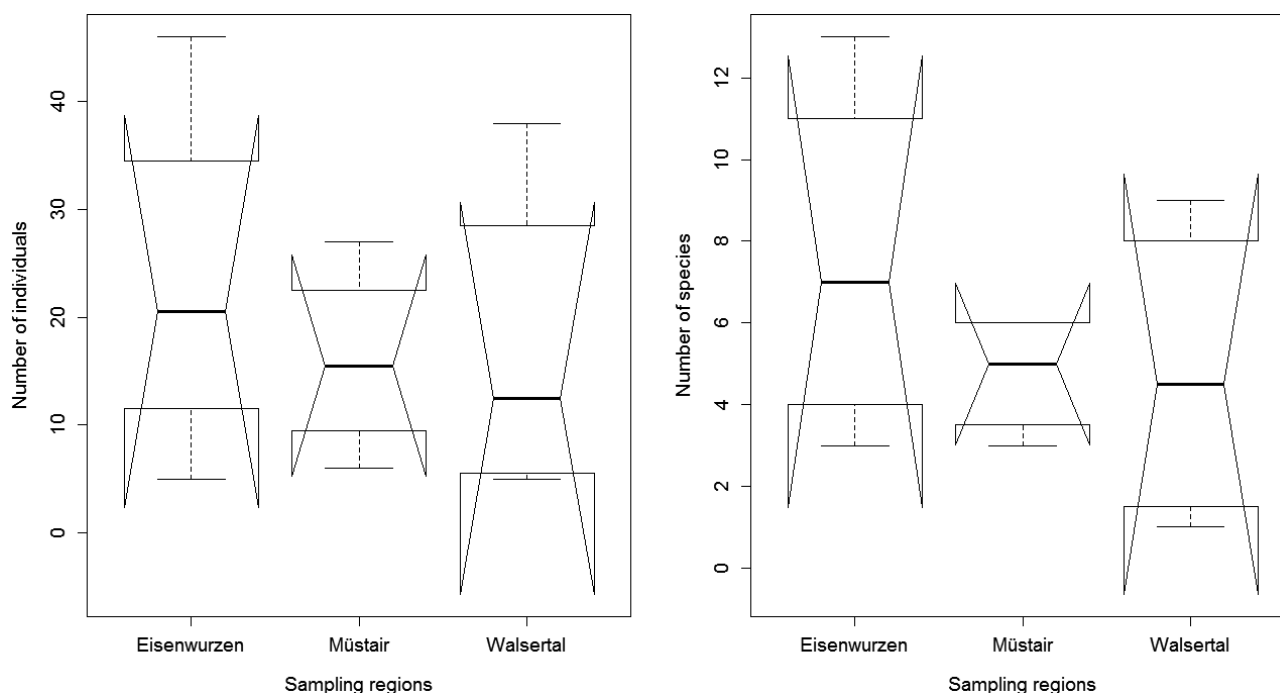
Fig. 1. Syrphid abundance and richness in three regions of Austria and Switzerland in 2015.

corded using these two methods were similar or differed significantly from each other.

MATERIAL AND METHODS

Study regions

This study was carried out in June and August 2015 in three regions in the Austrian and Swiss Alps (Table 1). The study sites were alpine meadows located in the regions of Eisenwurzen (Styria, Austria), Großes Walsertal (Vorarlberg, Austria) and Val Müstair (Graubünden, Switzerland) (Walcher et al., 2017). Altogether, six meadows (dominated by the grasses *Bromus erectus*, *Brachypodium pinnatum* and *Molinia caerulea*) with similar vegetation and located on south-facing slopes, were selected in each region ($6 \times 3 = 18$ meadows). The average size of the meadows ranged between 300 and 5150 m². There was no significant difference between the size of meadows in the three regions (ANOVA: $F = 2.27$, $p = 0.138$). Each meadow was surrounded by forest and an open landscape. At Eisenwurzen, the study region with a particularly high number of syrphids in 2015 (Fig. 1), the comparison of the two sampling methods was repeated in 2016 in order to strengthen the validity of the data.

Sampling methods

Syrphid species richness and abundance were surveyed using two different methods: line transects and observation plots. For the line transect method, three transects were established in the center of each study site. We made 30 sweeps per transect giving $3 \times 30 = 90$ sweeps per study site. Each transect was 15 m long and 2 m wide and the distance between each transect was 10 m. Sampling was carried out by experienced people and consisted of walking in straight lines with a sweep net (opening diameter 40 cm, 1 m bar length). Along with line transects, four 2 m² observation plots were established in a straight line at 0 m, 3 m, 9 m and 27 m distance per meadow. Observations were recorded over a period of 15 min for each plot. During the observations, every syrphid that visited a flower was recorded or collected using a sweep net if it was not possible to identify it on site. The frequency with which a sweep net was used in the observation plots depended on the number of unknown syrphids visiting a plot. There were four observation sessions (60 min per meadow) both in June and August 2015 when weather conditions were suitable, i.e. minimum temperature 15°C (temperature is a limiting factor for adult syrphid activity (Mudri-Stojnić et al., 2012), no rain or wind, dry vegetation and sunshine. The time of sampling was restricted to between 10 a.m. and 5 p.m. when syrphids are most active visiting flowers and was carried out by the same people, that is, they had the same experience of identifying syrphids. The two methods were carried out simultaneously in each meadow, therefore, field conditions during sampling were identical for both methods. Specimens that could not be identified were killed using ethyl acetate, transferred to plastic vials and brought to the

Table 1. Sites and their regional characteristics along an altitudinal gradient in Austria and Switzerland where Syrphids were sampled.

Country	Region	Municipality	Mean temperature in June and August	Mean annual precipitation	Altitude a.s.l.*	GPS coordinates
Austria	Eisenwurzen	St. Gallen Stainach Pürgg	19°C	1087 mm	660 ~ 790 m	47°41´N, 14°37´E 47°32´N, 14°06´E 47°31´N, 14°03´E
Austria	Großes Walsertal	Buchboden	22°C	1630 mm	1180 ~ 1250 m	47°14´N, 09°57´E
Switzerland	Val Müstair	Tschierv Valchava St. Maria	24°C	810 mm	1740 ~ 1800 m	46°36´N, 10°20´E 46°36´N, 10°24´E 46°36´N, 10°25´E

* above sea level

Table 2. Syrphid species recorded by the two sampling methods (observation plot and line transect method) in three regions in 2015. The numbers indicate the total number of specimens recorded.

Species	Region	Number of individuals	
		Observation plot	Line transect
Caliprobola speciosa	Großes Walsertal	1	
Cheilosia impressa	Val Müstair	1	
Cheilosia personata	Eisenwurzen		1
Chrysotoxum cautum	Großes Walsertal	1	
Chrysotoxum vernale	Großes Walsertal	1	
Epistrophe diaphana	Großes Walsertal	3	
Episyrphus balteatus	Eisenwurzen	7	1
	Val Müstair	2	
	Großes Walsertal	2	
Eristalis jugorum	Großes Walsertal	2	
Eristalis tenax	Eisenwurzen	1	1
	Val Müstair	1	
Eupeodes lapponicus	Eisenwurzen	18	2
	Val Müstair	1	
	Großes Walsertal	1	
Melanostoma mellinum	Eisenwurzen	2	4
	Val Müstair	9	11
	Großes Walsertal	13	10
Melanostoma scalare	Großes Walsertal	1	
Merodon equestris	Eisenwurzen	1	1
Myathropa florea	Eisenwurzen	1	
	Großes Walsertal	1	
Orthonevra geniculata	Eisenwurzen	1	
Parasyrphus annulatus	Val Müstair	1	
Pelecocera tricincta	Großes Walsertal	1	
Pipizella viduata	Eisenwurzen		1
Pipizella virens	Eisenwurzen		1
	Großes Walsertal	2	
Platycheirus albimanus	Val Müstair	1	
Platycheirus sticticus	Val Müstair	1	
Rhingia borealis	Eisenwurzen	1	
Scaeva pyrastri	Val Müstair	1	
Sphaerophoria interrupta	Eisenwurzen		1
	Val Müstair		2
Sphaerophoria scripta	Eisenwurzen	4	11
	Val Müstair	2	2
	Großes Walsertal	6	1
Sphegina sibirica	Eisenwurzen	2	
Syritta pipiens	Eisenwurzen	4	
	Val Müstair	1	3
Syrphus ribesii	Eisenwurzen	1	
Volucella bombylans	Eisenwurzen		1
	Val Müstair	2	1

Table 3. Syrphid species recorded using the two collection methods (observation plot and line transect method) in one region (Eisenwurzen) in 2016. The numbers indicate the total number recorded.

Species	Number of individuals	
	Observation plot	Line transect
Cheilosia impressa	1	1
Chrysotoxum bicinctum	6	
Chrysotoxum octomaculatum	1	
Episyrphus balteatus	6	
Eristalis tenax	1	1
Eristalis arbustorum	3	
Eristalis jugorum	1	1
Eristalis nemorum	1	
Melanostoma mellinum	24	3
Meliscaeva auricollis	1	
Merodon equestris		1
Merodon rufus	2	
Myathropa florea	4	
Paragus haemorrhous	1	
Parasyrphus lineolus	1	
Pipiza noctiluca	1	
Pipizella sp.	2	
Pipizella viduata	3	1
Pipizella virens	1	
Sphaerophoria interrupta	2	
Sphaerophoria scripta	33	7
Sphaerophoria sp.	1	
Syritta pipiens	4	
Syrphus ribesii	2	

pling month and region as random factors for the 2015 data, and month and year as random factors for the 2015 and 2016 data recorded at Eisenwurzen. We also verified normality (Shapiro test) and homogeneity of variance (Fligner test) in each model. Pearson correlations were computed to determine covariance in the structure of the data. We performed statistical analysis in R version 3.3.1 (R Core Team, 2016) and used an alpha level of 0.05.

To assess the difference in species assemblages recorded using the line transect and observation plots, a principal coordinate analysis (PCO) based on a resemblance matrix of Bray-Curtis similarity was carried out (Leyer & Wesche, 2007). A permutational ANOVA (PERMANOVA) was computed to test for significant differences in species assemblages recorded using the two methods (Anderson et al., 2008). SIMPER-routine (similarity percentages) was used to determine the role of individual species in contributing to the difference in the results recorded using the two sampling methods, which decomposes average Bray-Curtis dissimilarities between all samples into the percentage contribution from each species (Anderson et al., 2008). Time and region were included in the PERMANOVA model to account for the variations in the two sampling methods (Öberg et al., 2007). Measures of resemblance among different species assemblages were made using Bray-Curtis resemblance (Somerfield, 2008). SIMPER, PERMANOVA and PCO were done using the Primer

laboratory. They were preserved in 70% ethanol and identified using identification keys (Stubbs, 1983; Veen, 2010).

Data analysis

We checked the data for normal distribution graphically by creating QQ-plots. To evaluate differences in species richness and abundance we used linear mixed-effect models including sam-

Table 4. PERMANOVA table showing the effect of two sampling methods, month, region and interaction on syrphid species assemblages in 2015. Significant effects are shown in bold.

| Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Sampling method	1	2877.8	2877.8	3.3116	**0.0091**	9941
Month	1	3607.9	3607.9	4.1517	**0.0020**	9952
Region	2	5411.7	2705.9	3.1137	**0.0011**	9934
Month × Region	2	7154.5	3577.2	4.1164	**0.0002**	9924
Month × Sampling method	1	487.14	487.14	0.56055	0.7508	9952
Region × sampling method	2	2324	1162	1.3372	0.2037	9921
Month × Region × Sampling method	2	2259.5	1129.8	1.3	0.2297	9944

(a)

(b)
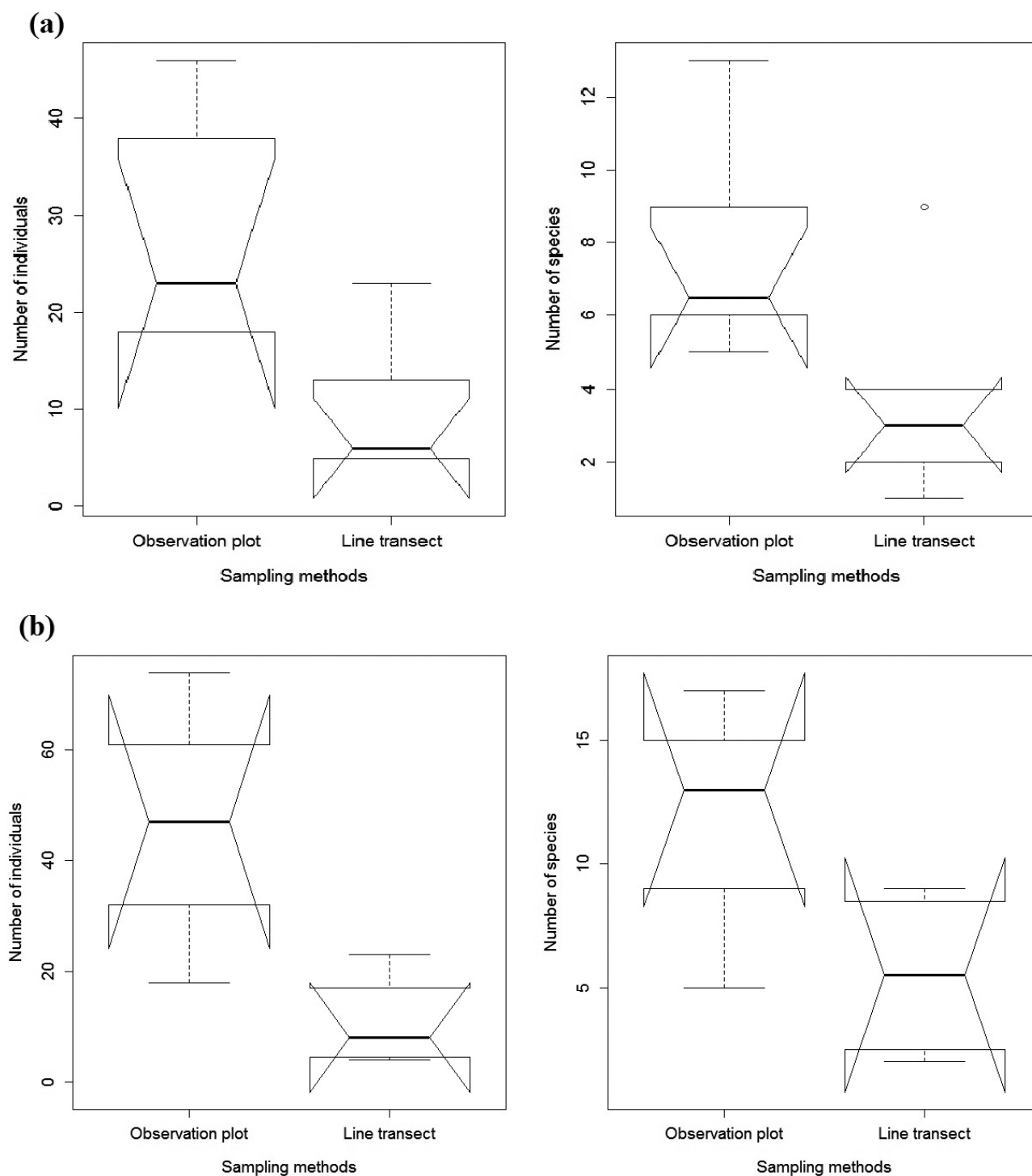
Fig. 2. Syrphid abundance and richness recorded by two sampling methods in (a) the three regions Eisenwurzen, Großes Walsertal and Val Müstair in 2015 and (b) one region Eisenwurzen in 2015 and 2016. Boxplots show the medians, the 25% and 75% percentiles and the 10% and 90% percentiles (indicated as dashed lines), and notches.

version 6.1.13 with PERMANOVA+ (PRIME-E Ltd., Plymouth, UK).

RESULTS

Overall, 361 individuals and 41 species belonging to 26 genera were recorded using the two methods over the course of two years. In 2015, a total of 224 individuals belonging to 29 species and 23 genera were recorded in the three regions. 26 species were recorded in the observa-

tion plots and 12 species along the line transects (Table 2). In 2016, 137 individuals belonging to 24 species and 16 genera were recorded at Eisenwurzen. 23 species were recorded in the observation plots and seven species along the line transects (Table 3).

A significantly higher abundance ($t = 3.174$, df = 1, p = 0.001) and richness of syrphids ($t = 3.076$, df = 1, p = 0.002) was recorded in observation plots than along the line transects in 2015 (Fig. 2a). *Eupeodes lapponicus* and
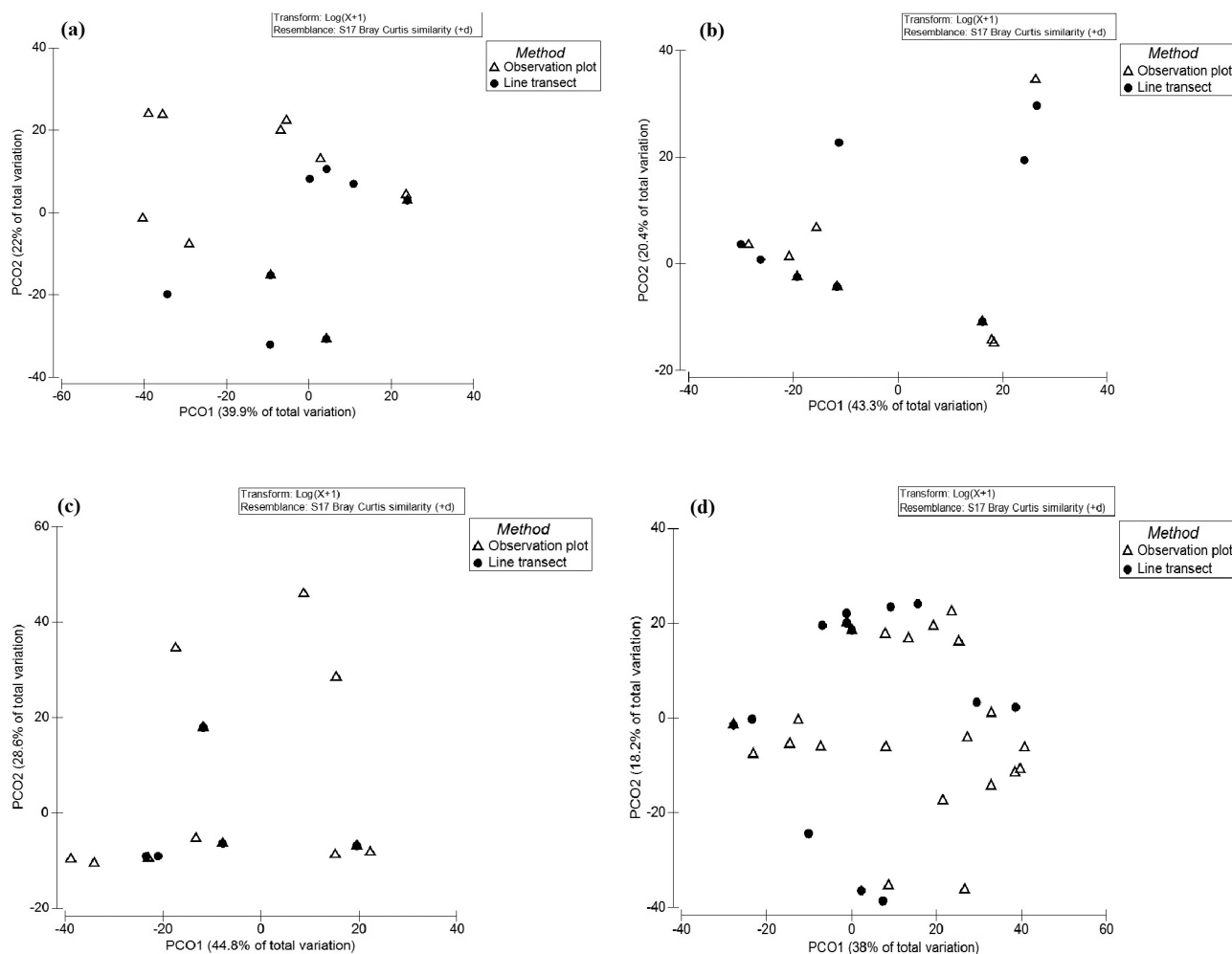
Fig. 3. Principal coordinate analysis (PCO) showing the distribution of syrphid species assemblages recorded by the two sampling methods (Observation plot and line transect) in the regions (a) Eisenwurzen, (b) Val Müstair and (c) Großes Walsertal in 2015. PCO showing species assemblages in one region (Eisenwurzen) in 2015 and 2016 (d).

Melanostoma mellinum were the most abundant species in 2015. Comparing data for one region (Eisenwurzen) in 2015 and 2016, revealed similar results (Fig. 2b). In addition, syrphid abundance ($t = 2.919$, df = 1, p = 0.003) and richness ($t = 2.118$, df = 1, p = 0.034) were significantly higher in the observation plots. Again, *M. mellinum* and, in addition, *Sphaerophoria scripta* were the most abundant species in 2016.

Significantly different species assemblages were recorded using the two sampling methods (Table 4, 5). Syrphid species assemblages in 2015 were significantly affected by sampling method, month and region (Table 4). There was also a significant interaction between month and region (Fig. 3a, b, c). Separation of species assemblages recorded using the two sampling methods was revealed by PCO for the region Eisenwurzen in 2015 and 2016 (Fig. 3d). In addition, there were significant effects of sampling method, month and year, including a significant interaction between month and year, on species assemblages in the region Eisenwurzen in 2015 and 2016 (Table 5).

Four species (*M. mellinum*, *S. scripta*, *Episyrphus balteatus* and *E. lapponicus*) accounted for 95.43% of the similarity in the species assemblages recorded for the observation plots and two species (*M. mellinum* and *S. scripta*) accounted for 96.22% of in the similarity in the records for the line transects. The average dissimilarity of 70.58%

Table 5. PERMANOVA table showing the effect of two sampling methods, months, year (2015 and 2016) and interaction on syrphid species assemblages in one region (Eisenwurzen). Significant effects are shown in bold.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Sampling method	1	8230.3	8230.3	8.0317	**0.0001**	9930
Month	1	5377.2	5377.2	5.2475	**0.0001**	9952
Year	1	4470	4470	4.3621	**0.0004**	9956
Month × Year	1	4021.6	4021.6	3.9245	**0.0020**	9936
Month × Sampling method	1	853.65	853.65	0.83305	0.5473	9944
Year × Sampling method	1	4555.4	4555.4	4.4455	**0.0010**	9945
Month × Year × Sampling method	1	853.08	853.08	0.83249	0.5475	9953

in the species assemblages recorded using the two methods was due differences in the incidences of five species (*S. scripta*, *M. mellinum*, *E. balteatus*, *L. lapponicus* and *Syritta pipiens*).

DISCUSSION

Our results show that the observation plot method is the best for determining abundance and richness of syrphids. Neither of the two methods recorded all the species but the observation plot method recorded more individuals and more of the species occurring in the study regions. During the periods of time (15 min) spent at each plot it is likely observer became part of the background and after a while syrphids were not disturbed by the collector and became active again. In contrast, continuous sweep netting along a line transect might have disturbed the syrphids and caused them to fly away.

Syrphids are very active pollinators and react quickly (Kühn et al., 2006). Species like *E. balteatus*, *Myathropa florea* and *Syrphus ribesii*, which were almost exclusively recorded in observation plots, tended to hover near the ground and fly through vegetation (Speight, 2014), where they could easily be observed but possibly not recorded by the line transect method. Differences in the syrphid assemblages between months and regions are usually interpreted in terms of environmental requirements (Hawkins et al., 2003), including temperature regimes and overwintering sites. For example, *S. ribesii* (Hart & Bale, 1997) is very tolerant of frost and the overwintering ability of *E. balteatus* is strongly related to restricted cold-hardiness (Hondelmann & Poehling, 2007). In addition, geographic environmental variables (altitude, latitude and longitude) are known to affect species assemblages (Keil & Konvicka, 2005) and may account for the significant regional effect recorded in 2015. Moreover, the significant method and year interaction indicates that both methods should be used if the aim is to collect as many species and individuals as possible.

Though the area sampled by the observation plot method ($4 \times 2 = 8$ m^2) was less than a tenth of that sampled by the line transect method ($2 \times 15 \times 3 = 90$ m^2), significantly more species and individuals were recorded by this method. Based on the current study, the observation plot method seemed to be better than the line transect method for several reasons. First, by using the observation plot method the observer only records the animal group of interest, i.e. syrphids. In contrast, in using methods like yellow pan traps, Malaise traps or the line transect method other insects are also killed, which is contrary to insect conservation (Costello et al., 2016). Second, the observation plot method is better in very sensitive ecosystems because smaller areas are disturbed by the survey.

Overall, this study should help in selecting the appropriate sampling techniques for assessing syrphids in alpine grasslands at different altitudes. The observation plot method was shown to be more appropriate in terms of recording more grassland species of syrphids and individuals. This method appears to be adequate for studies that aim to compare differences in syrphid numbers between various grassland habitats. The observation plot method is the best providing the observers have a good knowledge of syrphids and are able to identify them to species in the field.

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