MASTER'S THESIS

„Optimization of butterfly surveys in the context of long-term monitoring“

Master of Science in Ecology and Biodiversity at the University of Innsbruck

Department of Ecology

submitted by

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Summary

Butterflies are important indicator species in biodiversity monitoring and can be surveyed and identified relatively easily in contrast to many other insect groups. Therefore, long term monitoring of butterflies can give valuable information about the decline of insect species that can be observed worldwide. In order to use the available resources as efficiently as possible the number of surveys that are conducted per site and season and the temporal distribution of surveys should be considered carefully. The goal of this study was to find a number of surveys that allows the detection of an adequate percentage of surveys without spending too many resources on surveys that promise only little additional information gain and to find the optimal times during the season to conduct these surveys.

Seven sites in Tyrol were surveyed for butterflies nine times in between the end of May and the end of August 2019 using the same methods as in the Viel-Falter project (a butterfly monitoring program in the Austrian provinces Tyrol and Vorarlberg as well as in the Italian South Tyrol). The data was interpolated end extrapolated to obtain an estimate of the percentage of overall species richness that was detected after a certain number of surveys. Besides the information gain with each additional survey and the influence of the timespan in between surveys was regarded.

I set 80% of the estimated overall species richness as target value. This value was reached after twelve surveys on average. However, the information gain with each additional survey dropped below 5% of the overall species richness for more than five surveys. Conducting twelve surveys per site would mean to spend many resources on surveys with little information gain. Therefore, conducting five surveys per site – after which on average 58.50 % of the estimated overall species richness are detected - seems to be a reasonable recommendation to balance an efficient use of resources and the detection of a high number of species per site. The timespan in between surveys had an effect on the community similarity on the sites but there was no significant difference between randomly selected surveys and those obtained with a minimum timespan between consecutive surveys. There was no significant effect of vegetation height on seasonal changes in butterfly species richness. Species richness was significantly related to butterfly abundance indicating that most surveys should be when overall abundance is highest. For the sites studied here this would be in late summer. As there are species that only fly early in the season at least one survey should be conducted in early summer as well.

Zusammenfassung

Schmetterlinge sind wichtige Indikatorarten und können im Gegensatz zu anderen Insektengruppen relative einfach erhoben und bestimmt werden. Schmetterlingsmonitoring ist daher von großer Bedeutung – insbesondere in Zusammenhang mit dem globalen Biodiversitätsverlust. Ziel dieser Studie war es, ein solches Monitoring zu optimieren. Die Anzahl an Erhebungen, die pro Saison auf einer Fläche durchgeführt werden sollten, wurde dabei ebenso betrachtet, wie die idealen Zeiträume für die Erhebungen.

Hierzu wurden sieben Erhebungsflächen innerhalb Tirols jeweils neunmal zwischen Ende Mai und Ende August 2019 beprobt. Dabei wurden die gleichen Methoden wie im Viel-Falter Projekt (einem Monitoringprogramm für Schmetterlinge in Nord- und Südtirol sowie Vorarlberg) angewandt. Die Daten wurden extrapoliert, um eine Schätzung der Gesamtartenzahl auf den Erhebungsflächen zu erhalten. Es wurde dann berechnet, welcher Anteil der geschätzten Artenzahl jeweils nach einer bestimmten Anzahl von Erhebungen erfasst wurden und wie groß der Informationsgewinn mit jeder zusätzlichen Erhebung ist. Außerdem wurde untersucht, welche Bedeutung die Zeitspanne zwischen aufeinanderfolgenden Erhebungen hat.

Die 80% der Gesamtartenzahl, die in dieser Studie als angemessen angenommen wurden, wurden im Durchschnitt nach zwölf Erhebungen erreicht. Der Informationsgewinn mit jeder zusätzlichen Erhebung sank für mehr als fünf Erhebungen unter 5% der geschätzten Gesamtartenzahl. Zwölf Erhebungen pro Fläche durchzuführen würde demnach bedeuten, viele Ressourcen auf Erhebungen mit einem geringen Informationsgewinn zu verwenden. Daher erscheinen fünf Erhebungen pro Fläche – nach denen im Durchschnitt 58.50% der Gesamtartenzahl erfasst werden – als sinnvolle Empfehlung. Die Zeitspanne zwischen zwei Erhebungen hatte einen Einfluss auf die Ähnlichkeit der Artgemeinschaft, aber es konnte kein signifikanter Unterschied zwischen den Ergebnissen von zufällig verteilten Erhebungen und solchen mit einer minimalen Zeitspanne zwischen aufeinanderfolgenden Erhebungen gefunden werden. Die Höhe der Vegetation stand nicht in einem signifikanten Zusammenhang mit saisonalen Veränderungen der Schmetterlingsartenzahl. Es bestand ein signifikanter Zusammenhang zwischen der während einer Erhebung erfassten Abundanz und der Anzahl erfasster Arten. Daher wäre es sinnvoll, Erhebungen hauptsächlich im Spätsommer – der Zeit höchste Abundanz für die hier beprobten Flächen – durchzuführen. Da es auch Arten gibt, die nur zu Beginn der Saison fliegen, sollte trotzdem mindestens eine der Erhebungen im Frühsommer durchgeführt werden.

1 Introduction

1.1 Biodiversity loss

Since the middle of the 20th century there is a strong acceleration in the impact humans have on the planet. This great acceleration is coupled with a decline in biodiversity that can be observed in many different taxa including insects (Brooks et al. 2012; Habel et al. 2019; Hallmann et al. 2017). Lepidoptera are among the insect groups that are most affected by biodiversity loss (Sánchez-Bayo and Wyckhuys 2019). All across Europe the population numbers and distribution areas of butterflies are declining (Nilsson et al. 2013; Thomas et al. 2004; Wenzel et al. 2006) with higher losses in specialist species compared to generalist species (van Swaay et al. 2006). The main reasons for these declines are land use changes that lead to loss and degradation of habitats (Thomas 2016). In Europe semi-natural landscapes that are shaped by traditional farming methods are especially valuable for butterflies (van Swaay et al. 2006). These semi-natural landscapes are decreased by two oppositional developments: the intensification and the abandonment of agricultural land (Habel et al. 2019). Other drivers that threaten butterfly (and other insect) biodiversity are pollutants, invasive species and climate change (Sánchez-Bayo and Wyckhuys 2019).

1.2 Butterflies as surrogate species

The term surrogate species is used here as described by Caro and O'Doherty (1999) who distinguish different kinds of surrogate species: indicator, umbrella and flagship species. Indicator species are used to assess environmental conditions or characteristics of other species that would otherwise be difficult or expensive to measure. Umbrella species are target species in conservation biology whose protection is beneficial for a range of other species. Flagship species are charismatic species that are used to rise public attention.

Butterflies are widely used as ecological and environmental indicator species. There are many examples of butterflies as indicators for the effects of land use change (e.g. Bobo et al. 2006; Brown and Freitas 2000; Herrnando et al. 2016), climate change (e.g. Stuhldreher and Fartmann 2018; Wilson et al. 2007) and restauration measures (e.g. Kleintjes et al. 2004; Rákosy and Schmitt 2011). Due to their short lifecycle they respond more rapidly to environmental factors compared to many other taxa (Thomas et al. 2004). Butterflies are especially important as indicators when it comes to the decline in insects. The high number of insect species worldwide makes it impossible to measure the development of their population numbers directly. Another difficulty in the monitoring of insects is our lack of knowledge about many taxa and that they are often difficult to identify (Conrad et al. 2007). Butterflies are relatively easy to survey and identify and are therefore considered to be a taxon that can be monitored with adequate effort and precision in many parts of the world (Thomas 2005). Butterflies are regarded as reasonable representatives of many other invertebrates in terrestrial ecosystems (Fleishman and Murphy 2009; Thomas 2005). However, their value as indicator species is also seen critically. They might react too responsive to short term changes in ecological conditions to connect their population changes to environmental trends. This is especially true for data derived from short time periods while data from long-term monitoring programs can allow to draw meaningful conclusions about environmental changes such as climate change (Fleishman and Murphy 2009). Even when long-term data is used the value of butterflies as indicator species should be considered carefully as it depends on the scale, the region and the taxonomic groups under comparison (Gerlach et al. 2013). Next to their importance as indicator species butterflies are very suitable as flagship and umbrella species. Due to their colorful and charismatic appearance they are perceived more positively by school children and adults than other insect groups (Barua et al. 2012; Breuer et al. 2015; Schlegel and Rupf 2010). Therefore, they can attract public attention and raise awareness for the importance of nature conservation in general and the conservation of invertebrate species in particular (New 1997; Spitzer et al. 2009). Due to these characteristics, butterflies are often monitored in citizen science projects (Schmeller et al. 2009).

1.3 Butterfly monitoring

Even though butterflies are one of the best studied groups of insects we still know little about them in comparison to vertebrate species (Lewis and Senior 2011). Because of the declining numbers of many butterfly species and the significant role they play as surrogate species it is highly important to monitor the development of their population sizes and their distribution. As already mentioned, long term monitoring programs are essential to detect meaningful trends in butterfly abundance and species richness. The first European butterfly monitoring program started in the UK in 1976 (Pollard 1977). Afterwards monitoring programs were established in different European countries (van Swaay et al. 2008). Many of these programs use the transect based method that was established by Pollard and Yates (1995). Even though long-term monitoring programs are highly important in ecological research (Lovett et al. 2007), the resources available for such programs are often limited. Many of the butterfly monitoring programs rely on the involvement of volunteers to obtain the necessary data at low costs. Next to the supply of observation data this approach also has the advantage to raise public awareness for conservation topics and to increase the acceptance of nature conservation measurements (Toomey and Domrose 2013; Wang et al. 2018). In order to get high quality results on species level it is still necessary to have surveys done by experts that complement the data derived from the observations made by volunteers.

1.4 Optimization of survey effort

There always is a trade-off between the number of survey sites that can be observed and the intensity of surveying on the single sites. A lower number of sites with many surveys per site lead to detailed information about the individual site but might lack the necessary spatial coverage of the monitored region. On the other hand, surveying many sites allows to collect information on a finer spatial scale but comprises the risk to underestimate abundance and species richness per site too much. The number of surveys per site should be considered carefully to use the available resources as efficiently as possible.

Roy et al. (2007) determined the most efficient combination of number of survey sites and numbers of surveys per site to detect changes in the abundance of twenty widespread butterfly species with the Pollard Walk method used in the United Kingdom Butterfly Monitoring Scheme (UKBMS) (Pollard and Yates 1995). They analyzed the statistical power of different monitoring schemes and came to the conclusion that three visits during this time period are most efficient in the context of the UKBMS. Power analysis as a method to analyze the most effective number of surveys is also used to analyze the influence of environmental factors such as transgenic crops on butterfly abundance (Lang 2004; Lang and Bühler 2012). Another approach to the question about the ideal number of surveys is based on the number of species that is detected on the individual survey sites. This approach is especially useful if the focus of a monitoring program is not only on the overall changes in butterfly abundance but also on the detection of rare and threatened species that are more likely to be overlooked in reduced effort schemes. Assessment of Butterfly monitoring schemes in Sweden, Italy and Great Britain analyzed the percentage of detected butterfly species to determine an adequate number of surveys per site and season (Dennis et al. 1999; Hardersen and Corezzola 2014; Jonason et al. 2010; Wikström et al. 2009). The studies differ in the method chosen for surveying: while some are based on the Pollard walk method others are plot-based or use a combination of both approaches. Even those programs that use a transect-based approach differ in length and layout of the transects as well as in the time spent on each survey. Jonason et al. (2010) came to the conclusion that four or five visits are necessary in the context of the Swedish monitoring program NILS (The National Inventory of Landscapes in Sweden) to detect two thirds of the species and that more than six visits don’t add enough information to justify the additional effort. Similarly, Wikström et al. (2009) found that the number of detected species increased strongly in between one, two and three visits while the difference for more than five visits was less pronounced. After 6 visits 89% of the estimated overall species richness were detected in their study compared to 64% to 91% detected by Jonason et al. (2010) after the same number of visits. Hardersen and Corezzola (2014) found differences in between sites in their study region in northern Italy regarding the proportion of estimated species richness detected after nine surveys. While it was 90% for lowland sites only 70% of the estimated overall species richness were detected in the mountain sites. These differences in between sites from the same study show that even within the same monitoring region the conditions can vary substantially and affect the effectiveness of butterfly monitoring.

1.5 Temporal distribution of surveys

Next to the number of surveys that are conducted during the season the time when surveys are conducted can also influence the efficiency of butterfly monitoring. Different butterfly species occur during different times of the year (Fillecia et al. 2015; Roy and Sparks 2000). Univoltine species only have one generation and some of them are restricted to certain times of the season. An example is *Anthocharis cardamines* which only flies in spring and early summer while *Minois dryas* can only be observed in late summer (Stettmer et al. 2007). Other species have two generations each year. Most of these bivoltine species have one generation in early summer and a second one in late summer. Multivoltine species with more than two generations per season have overlapping generations and can be observed during the whole season. Most monitoring schemes consider the consequential changes in species composition during the season. In the British Butterfly monitoring scheme butterfly counts are conducted weekly during the season to ensure a good detection of species with different phenology (Pollard and Yates 1995). Other monitoring programs with reduced monitoring schemes implement regulations to ensure that surveys are spread evenly over the season. Hardersen and Corezzola (2014) found that the number of detected species for reduced effort schemes was higher when the surveys were spread evenly over the season compared to a random distribution.

Not only the species composition but also overall abundance and species richness change during the season. Roy et al. (2007) analyzed the abundance of twenty widespread butterfly species in the UK. They came to the conclusion that most bivoltine species in their stusy area have a higher second peak during late summer and that most univoltine species are present during this time in high abundances as well. Their recommendation is to focus surveys on July and August when abundance is highest and monitoring of these species is therefore most efficient.

The abundance of butterfly species does not only depend on butterfly phenology but also on human activity. This aspect is especially important for species that inhabit meadows which are mown during the season. Butterfly abundance is related to flower abundance and mean vegetation height during the season (Milberg et al. 2016). Not only the overall abundance but also the seasonal dynamics of butterfly abundance are connected to the mowing regime on a site. Butterfly abundance was shown to decrease directly after mowing and to increase again afterwards when the vegetation on a site regrows (Bruppacher et al. 2016). Hence, it might be advisable to conduct surveys when vegetation is high and to avoid surveying when a meadow is freshly mown.

1.6 The Viel-Falter Project

Viel-Falter is a monitoring program in a butterfly monitoring program in the Austrian provinces Tyrol and Vorarlberg as well as in the Italian South Tyrol for butterflies of the superfamily Papilionoidea. Monitoring in Tyrol started in 2018, in South Tyrol in 2019 and in Vorarlberg in 2020. Data is collected in open habitats by laypeople and by experts. In North Tyrol there are 100 survey sites with a defined transect of 50m. The volunteers survey all butterflies along this transect with the Pollard walk method (Pollard and Yates 1995). They use a simplified assessment scheme that was developed with school children (Rüdisser et al. 2017). In this scheme butterflies are divided into phenotypically similar groups that can easily be identified in the field. Each year 25 of the sites are visited four times during the season (end of May until the beginning of September) by experts who make a survey on species level of the area around the transect (more detailed information about the survey method can be found in chapter 2.1). The goal of the project is to detect changes in overall butterfly abundance in the project region as well as changes in the abundance of rare and threatened species. Changes in overall butterfly abundance can give valuable information about the impact of changing environmental conditions that are causes by e.g. land use and climate change not only on butterflies but also for other species for which butterflies are suitable indicators. On the other hand, monitoring of rare species is important under the aspect of species conservation especially in the context of species extinctions that occur worldwide. Data from monitoring programs such as the Viel-Falter program give valuable information that is needed for protection of these species.

1.7 Aim of the study

The aim of this master thesis was to optimize the butterfly monitoring scheme of the Viel-Falter project.

In order to determine the optimal number of surveys per site per season the following two research questions were investigated:

- **How many surveys are necessary to detect 80% of the overall species richness on a site?**
- **How much information (percent of overall richness, numbers of species) is gained with additional surveys?**

Beside the ideal number of surveys, it might play a role, how the surveys are temporarily distributed. To test whether the time in between consecutive surveys influences the efficiency of butterfly monitoring programs the following two research questions were addressed:

- **How is compositional dissimilarity of surveys on the same site related to the number of days that lie in between them?**

- **Is there a difference regarding the number of detected species in between surveys that are randomly distributed over the season and those with a defined timespan in between two consecutive surveys?**

To gain further insight into a timing of surveys that allows to detect a high number of species the influence of butterfly abundance and vegetation height were taken into account:

- **Is the number of detected species during a survey related to butterfly abundance?**
- **Does vegetation height influence the species richness on a survey site?**

2 Material and Methods

2.1 Surveys

Butterfly surveys were conducted on seven sites in Tyrol that are part of the Viel-Falter project. Five are close to Innsbruck and two near Steinach am Brenner (fig. 1 and 2). All sites are meadows and close to settlements (fig. 3 and 4) with an elevation from 659 m a.s.l. to 1123 m a.s.l.. Different types of meadows most of which were managed intensively or moderately intensively were surveyed (table 1).

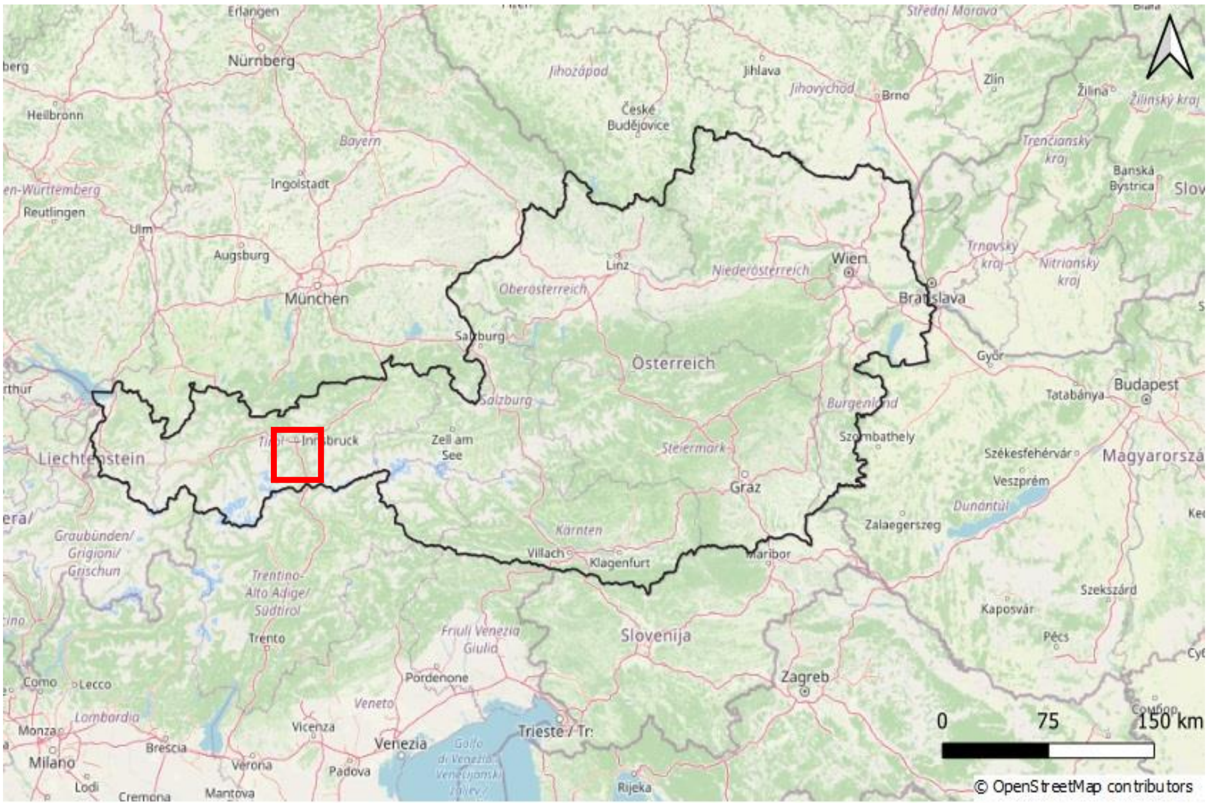

Figure 1 Location of the study region.

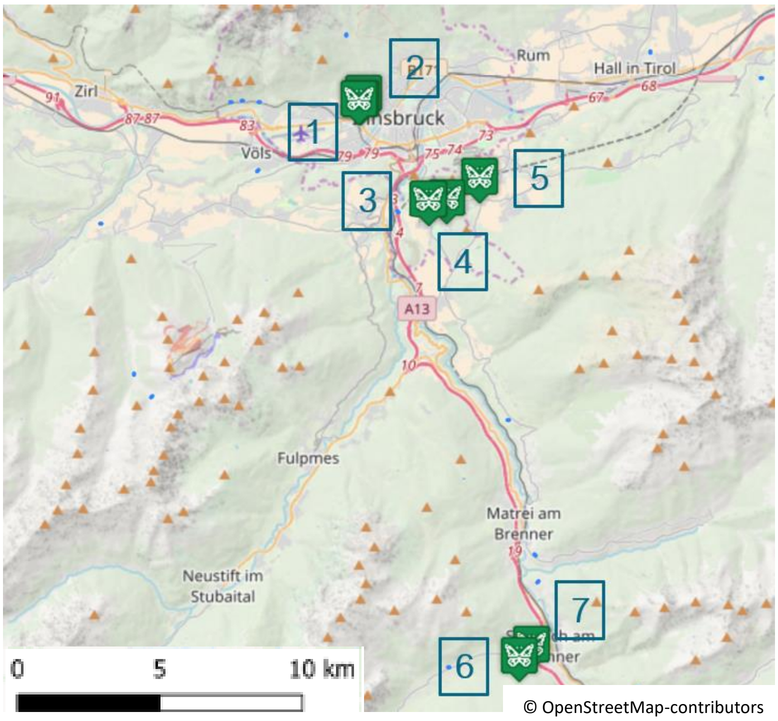

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Figure 3 Map with the Survey sites; 1: Butterbichl, 2: Schwabeneck, 3: Bachgang, 4: Lanser See, 5: Mühlsee-Wiese, 6: Scheiberbrücke, 7: Pflutschwiese

Figure 2 Aerial pictures of the transects; A: Butterbichl (left) and Schwabeneck, B: Bachgang (left) and Lanser See, C: Mühlsee-Wiese; D: Scheiberbrücke (left) and Pflutschwiese, from the Viel-Falter Homepage (https://viel-falter.at/cms/karte/, 02.01.2020)

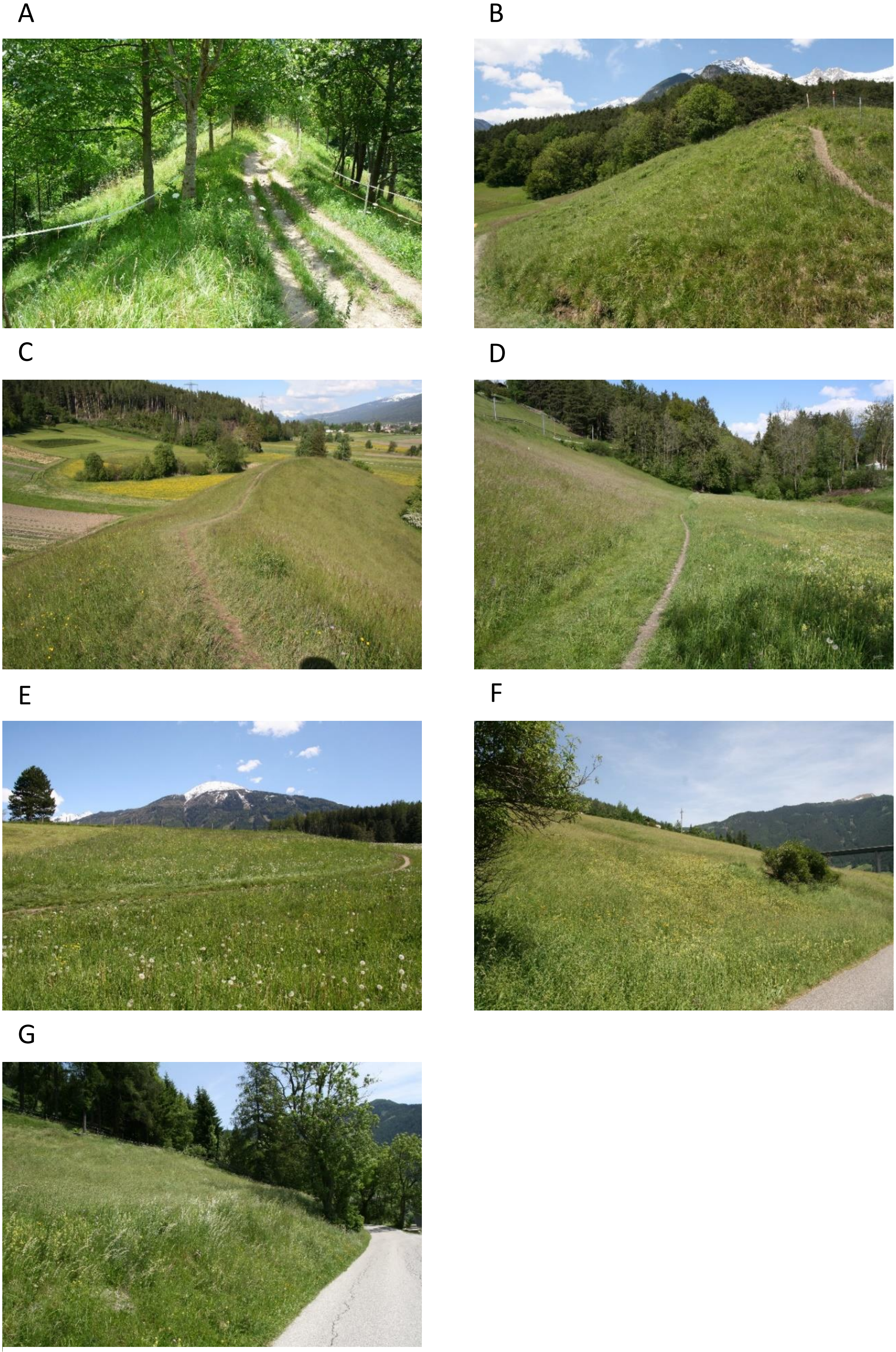

Figure 4 Pictures of the survey sites – A: Schwabeneck, B: Butterbichl, C: Bachgang, D: Mühlsee-Wiese, E: Lanser See, F: Scheiberbrücke, G: Pflutschwiese

Table 1 Elevation above sea level, vegetation and management intensity of the survey sites (Schwienbacher 2015)

SURVEY SITE	ELEVATION A.S.L.	TYPE OF VEGETATION	MANAGEMENT
BUTTERBICHL	690 m	dry meadow	moderately intensive
SCHWABENECK	725 m	moderately dry fallow	extensive
BACHGANG	838 m	semi dry grassland	extensive
LANSER SEE	845 m	fresh meadow	intensive
MÜHLSEE-WIESE	800 m	dry meadow	moderately intensive
SCHEIBERBRÜCKE	1080 m	moderately wet meadow	intensive
PFLUTSCHWIESE	1140 m	fresh meadow	moderately intensive

The surveys followed the survey protocol of the Viel-Falter project: surveys were conducted in between 10am and 5pm, on warm (above 13°C) and sunny days with low wind speed. At the two sites at Steinach am Brenner the regularizations regarding the windspeed (it should not be higher than two at the Beauford scale) could not always be met. Here the windspeed was in general higher than on the other sites and surveys were conducted at windspeed of up to three on the Beauford scale. Butterfly abundance was very likely not much influenced by this: wind speed was normally high on these sites and Wikström et al. (2009) showed that a wind speed of up to five on the Beauford scale did not influence the abundance of butterflies in their study. The height of the vegetation, the windspeed and the cloud cover were recorded at each survey. For each site a transect with a length of 50m is defined (see fig. 2 for the exact location of the transects). A timed survey of 30 minutes was conducted on an area of 1000m^2 - 10m on both sides or 20m on one side of the transect. The recording of the survey time was paused for the time it took to identify a butterfly. Butterflies that could not be identified by sight were caught with a butterfly net and those that could not be identified in the field were killed with ethyl acetate and identified by genital analysis. Species that can only be distinguished with DNA analysis were treated as one species. This was the case for *Colias hyale* and *Colias alfacariensis* and for *Leptidea sinapis* and *Leptidea juvernica.* The field guide "Tagfalter Bayerns und Österreichs" (Stettmer et al. 2007) was used for butterfly identification. Each site was surveyed nine times in between the end of May and the end of August. It was tried to conduct the surveys with at least a week in between them. This was not always possible on the sites Pflutschwiese and Scheiberbrücke because of unsuitable weather conditions.

2.2 Data Analysis

2.2.1 Software

R version 3.6.1. (R Core Team 2019) was used for data analysis. The following R packages were used in addition to those mentioned in the following chapters for data preparation, analysis and visualization: ggplot2 (Wickham 2019), janitor (Firke 2019), openxlsx (Walker 2019) and reshape2 (Wickham 2007).

2.2.2 Rarefaction and Extrapolation

Rarefaction is an interpolation method with which the average number of species after a certain number of samples or detected individuals can be determined. It is often used to allow a comparison of sample sites were the number of sampled individuals or the sampling intensity differs (Gotelli and Colwell 2001). Such a comparison is difficult otherwise as the relationship between sampled individuals/the number of samples and the detected species is not linear (Gotelli and Colwell 2011). A rarefaction curve is the smoothed average of a species accumulation curve and can be either sample- or individual based (fig. 6). A sample-based curve is used if the individuals were not sampled independently from each other and preserves the spatial or temporal structure of the data (e.g. aggregation or segregation).

The number of detected species rises steeply in the beginning as the probability that an added individual belongs to a new species (or that a sample contains new species) is high. The curve gets flatter when more species are added until it reaches an asymptote when the total species number is reached. (Gotelli and Colwell 2001). In most cases the assessed number of species is not close enough to the real species number to reach an asymptote. If there is a sufficient amount of data a rarefaction

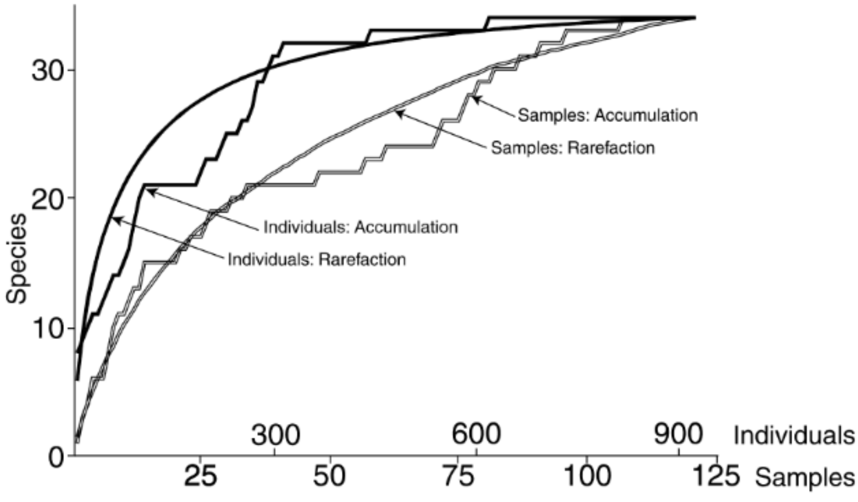

Figure 5 Species accumulation and rarefaction curves, Gotelli and Colwell (2001)

curve that does not reach the asymptote can be extrapolated in order to estimate the total species richness (Colwell et al. 2012).

The R package "iNEXT: iNterpolation and EXTrapolation for species diversity" (Hsieh et al. 2020) was used to for the interpolation and extrapolation of the data. The function iNEXT() allows the calculation of rarefaction and extrapolation curves for Hill numbers of order q = 0, 1 and 2 (species richness, Shannon diversity and Simpson diversity) for abundance- and incidence-based data. As the individuals were sampled in timed surveys and are therefore not independent from each other sample-based rarefaction and extrapolation curves were calculated using the presence- or absence-data of species in the different surveys. As the number of species is of interest in this study the curves were calculated for species richness (Hill numbers with q = 0).

For interpolation and extrapolation of the data a Bernoulli product model is assumed for incidence-based data. This approach is reviewed in Colwell et al. (2012). The data is organized in a species-by-sampling unit-incidence matrix W. The number of rows is equal to the number of observed species (S) and the number of columns is equal to the number of sampling units (T). W_{ij} = 1 if the ith species was observed in the jth sampling unit and Wij = 0 otherwise. The row sum $Y_i = \sum_{j=1}^{T} W_{ij}$ equals the number of sampling units in which the ith species was observed. Q_k defines the number of species that were observed in k sampling units. Hence, the number of observed species $S_{obs} = \sum_{k=1}^{T} Q_k$. Q_1 denotes the number of species that occurred only in one sample (unique species) and Q_2 denotes those species that occurred in two samples (duplicate species). If we assume that W_{ij} is a Bernoulli random variable with $\theta_i = P(W_{ij} = 1)$ then the probability distribution for the incidence matrix is defined as:

$$P(W_{ij}; i = 1, 2, \ldots, S; j = 1,2, \ldots, T) = \prod_{j=1}^{T} \prod_{i=1}^{S} \theta_i^{W_{ij}} (1 - \theta_i)^{1-W_{ij}} = \prod_{i=1}^{S} \theta_i^{y_i} (1 - \theta_i)^{T-y_i} \quad (1)$$

Based on the Bernoulli distribution described above an estimate of the expected species number in a random set of t samples $S_{sample}(t)$ can be derived. The minimum variance unbiased estimator is

$$\tilde{S}_{sample}(t) = S_{obs} - \sum_{Y_i > 0} \left[\binom{T - Y_i}{t} \Big/ \binom{T}{t} \right] \quad (2)$$

This formula was first derived by Shinozaki (1963).

For extrapolation of the Bernoulli distributed data an estimator of the number of species that are not detected ($\hat{Q}_0$) or of the asymptotic species richness (S_{est}) is required. The Chao2 estimator is a nonparametric minimum estimator of total species richness which incorporates a correction for small sample sizes (Chao 1987). It is defined as

$$\hat{Q}_{0\,Chao2} = [(T-1)/T][Q_1^2/(2Q_2)] \quad \text{for } Q_2 > 0 \tag{3a}$$

or

$$\hat{Q}_{0\,Chao2} = [(T-1)/T]\big[Q_q(Q_1-1)/\big(2(Q_1+1)\big)\big] \quad \text{for } Q_2 = 0 \tag{3b}$$

The estimated full richness of the assemblage is S_{est} = S_{obs} + $\hat{Q}_0$.

Chao et al. (2009) derived an estimator for extrapolation of sample-based rarefaction curves. It calculates the estimated expected number of species that are observed with T + t* sampling units (t* > 0).

$$\begin{aligned} \tilde{S}_{sample}(T+t^*) &= S_{obs} + \hat{Q}_0\left[1-\left(1-\frac{Q_1}{Q_1+T\hat{Q}_0}\right)^{t^*}\right] \\ &\approx S_{obs} + \hat{Q}_0\left[1-\exp\left(\frac{-t^*Q_1}{Q_1+T\hat{Q}_0}\right)\right] \end{aligned} \tag{4}$$

As sample sizes are small in this study, the Chao2 estimator is suitable as a minimum estimator of asymptotic species richness. While the extrapolation for Hill numbers of order q > 0 is nearly unbiased the extrapolation of species richness is considered reliable for up to the double of the taken samples (Chao et al. 2014). Therefore, the data was extrapolated to 18 surveys. The standard error and a 95% confidence interval were obtained with a bootstrapping method developed by Chao et al. (2014). The variance derived with this method is not conditional on the reference sample. This has the advantage that the variance is not 0 for the maximum number of samples taken (as here all the reference data is included and the species number is fixed) and the confidence intervals for rarefied and extrapolated data merge smoothly.

2.2.3 Information gain of additional surveys

Two different measures for the information that is gained with additional surveys were used. The mean number of new species with additional surveys was calculated and the mean percentage of the overall species richness that were gained with additional surveys.

2.2.4 Compositional dissimilarity

Measures of compositional (dis)similarity for abundance-based data compare the relative abundances of the species in two (or more) assemblages. The species composition of the samples taken on the same survey site were compared pairwise using the Horn index (Horn 1966). Let S be the total number of species in the combined assemblage and p_{i1} and p_{i2} the relative abundance of the ith species in the first and second sample respectively. Then the Horn index is defined as

$$S_H = \frac{1}{log2}\sum_{i=1}^{S}\left[\frac{p_{i1}}{2}\log\left(1+\frac{p_{i2}}{p_{i1}}\right)+\frac{p_{i2}}{2}\log\left(1+\frac{p_{i1}}{p_{i2}}\right)\right] \tag{6}$$

This index fulfills the requirements for similarity/dissimilarity measures that are described by Jost et al. (2011): density invariance, replication invariance and monotonicity. While the closely related Morisita-Horn index is more robust and resistant to undersampling as it is dominated by the more abundant species in an assemblage the Horn index is more sensitive to rare species. As the detection of rare and threatened species is of importance in the Viel-Falter project the Horn index was chosen for this study. The compositional dissimilarity between surveys was calculated with the function vegdist() from the R package "vegan: Community Ecology Package. R package version 2.5-6" (Oksanen et al. 2019).

Linear models with the dissimilarity of the surveys as respondent and the days in between surveys as explanatory variable were computed to analyze the relationship between these two variables. The following transformations were tested to find the one that best fits the data: simple linear, polynomial of degree two, square root, logarithmic and reciprocal. This was done for the single sites as well as for pooled data from all sites.

2.2.5 Temporal distribution of surveys

Rarefaction assumes that the samples are independent from each other and takes all possible combinations of surveys into account. This approach could underestimate the detected species richness as samples taken with only a short time span in between are likely to share more species than those further apart. Therefor rarefied species richness was compared to the number of species that are detected, when rules regarding the time span in between surveys are imposed.

For this comparison another approach for rarefaction of the data was used. Next to the already described method that is based on combinatorial equations it is also possible to obtain a rarefaction curve by random resampling of the surveys (Gotelli and Colwell 2011). For this purpose, the R package "vegan" was used. The rarefied species richness for samples sizes $n < m$ was calculated by repeatedly resampling n out of m samples without replacement with 50 permutations using the function specaccum().

The results were compared with those obtained with combinations of surveys that were more evenly distributed over the sampling season. For this purpose, a minimum number of days in between surveys was defined. For two surveys there had to be at least 30 days in between the surveys. For three and four surveys the timespan was reduced to 21 days (same as in the Viel-Falter project) or 18 days when there were too few combinations with 21 days in between each survey. The timespan for five, six and seven surveys had to be 15, ten, eight days respectively. For one and eight surveys all possible combinations were taken into account.

The variance that is obtained with the two methods is conditional on the reference sample and therefore approaches 0 when the number of samples tends to the maximum number of taken samples. This facilitates the comparison of the results and is the reason why a method for rarefaction was chosen that differs from the one already used.

2.2.6 Influence of abundance and vegetation height

The influence of abundance and vegetation height on the detected species richness during a survey were analyzed using simple linear regression with abundance or vegetation height on a survey day as explanatory and the number of observed individuals as respondent variable. Regression analysis was conducted for each site individually and for the combined data from all sites. For the combined test the data on abundance and vegetation height was scaled using min-max normalization:

$$z_i = \frac{x_i - \min(x)}{\max(x) - \min(x)} \tag{7}$$

This way it was avoided that effects of mean abundance or mean vegetation height of a site on the species richness influence the results.

3 Results

In total 1104 butterflies that belonged to 46 species were detected on the seven survey sites. The sites where the lowest number of species was detected was Schwabeneck with 12 species and the one with the highest species number Pflutschwiese with 34 species. Butterfly abundance ranged from 80 individuals at Lanser See to 214 at Pflutschwiese (fig. 7). The mean number of species per site was 20.43 (standard deviation: 7.96) and the mean abundance 157.57 (standard deviation: 46.32). Species lists for the individual sites can be found in the supplement (table S1).

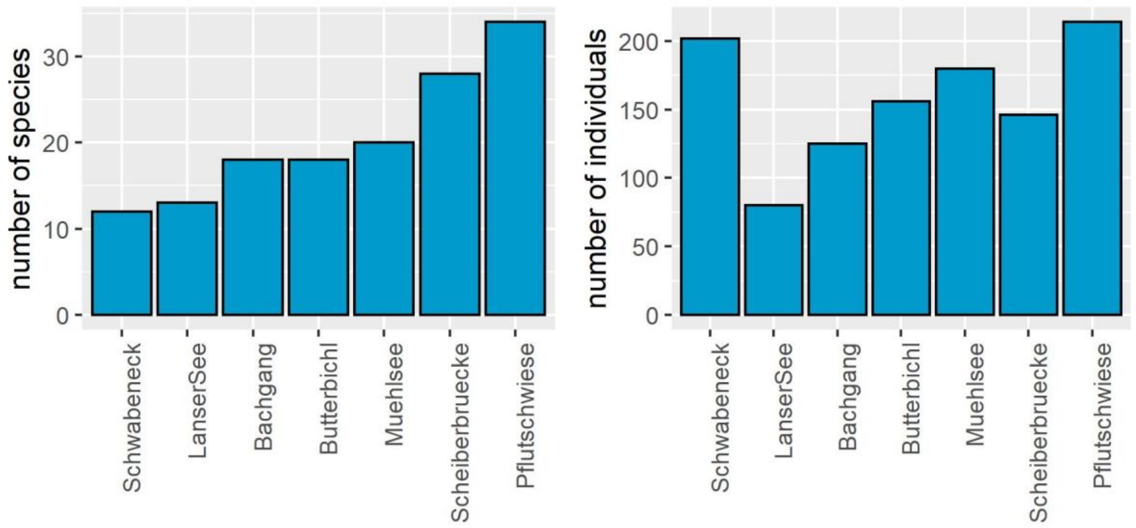

Figure 6 Number of detected species and individuals for the survey sites of this study.

Species richness and butterfly abundance on the sites changed during the season. On most sites there were two peaks with higher abundance and species richness and a period with low numbers in between (fig. 8). This pattern is also reflected by the curves with the means of the standardized values for abundance and species richness derived from the pooled data of all sites (fig. 9). On most sites the butterfly abundance and the species richness follow a similar pattern. One exception is the high abundance of butterflies at the end of the season at Schwabeneck and the relatively low number of species during the same period. The reason for this discrepancy is the appearance of *Minois dryas* in high abundances on that site. Vegetation height decreased once or twice during the season indicating that the sites were mown during that time (fig. 8).

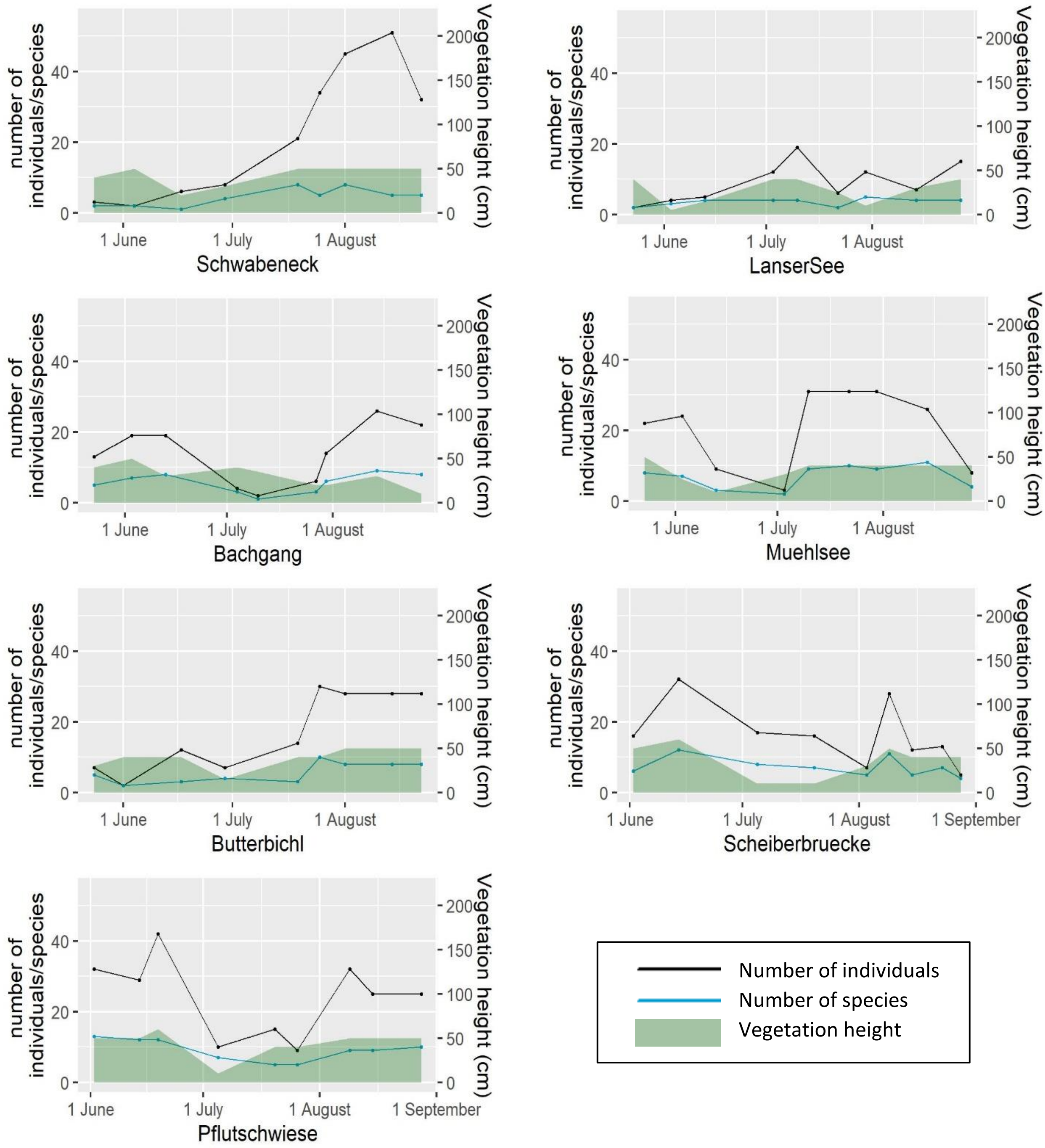

Figure 7 Numbers of individual butterflies and of butterfly species and vegetation height on the survey sites during the season

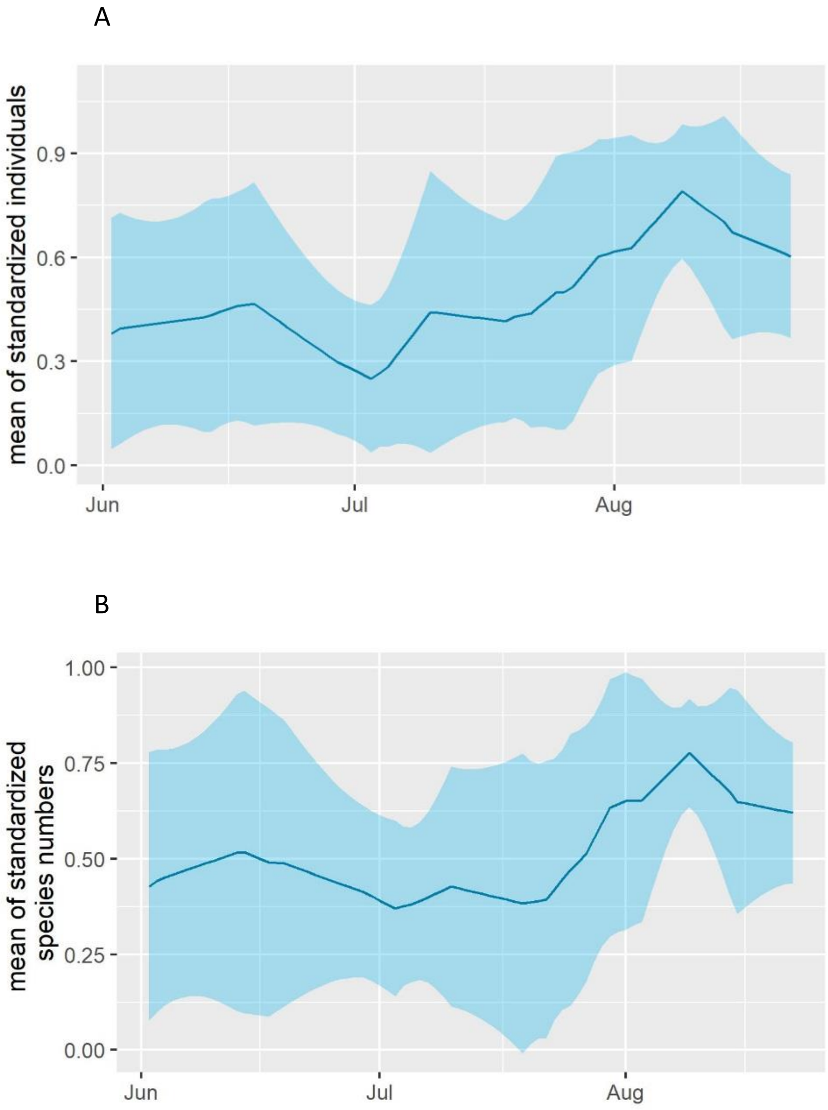

Figure 8 Mean of standardized abundance (A) and species richness (B) over the season with standard deviation. The values for the single sites were interpolated linearly and standardized using min-max normalization.

3.1 Rarefaction and extrapolation

The estimated overall species richness obtained with the Chao 2 estimator ranged from 15.37 to 48.22. The standard error is high for some of the survey sites leading to a broad 95% confidence interval. For the site Mühlsee, where the standard error is highest the 95% confidence interval ranges from 23.52 to 112.033 (table 2).

Table 2 Observed species number after nine surveys and the estimated overall species richness for the survey sites with standard error and the upper and lower boundaries of the 95% confidence interval

SURVEY SITE	OBSERVED	ESTIMATOR	SE	95% LOWER	95% UPPER
SCHWABENECK	12	16.00	6.43	12.43	48.62
LANSER SEE	13	15.37	3.14	13.33	30.05
BACHGANG	18	20.78	3.30	18.44	35.46
BUTTERBICHL	17	27.89	11.74	18.95	77.91
MÜHLSEE	20	38.00	18.00	23.5	112.03
SCHEIBERBRÜCKE	28	34.72	5.37	29.70	54.62
PFLUTSCHWIESE	34	48.22	9.49	38.33	80.73

Some of the rarefaction and extrapolation curves start to flatten at the end, which indicates that they come near the asymptote (e.g Lanser See and Bachgang) other curves like those for Mühlsee and Butterbichl are still rising relatively steeply at the extrapolated value for 18 surveys (fig. 10).

While the extrapolated species richness for some sites (Lanser See, Scheiberbrücke and Bachgang) is close to 100% of the estimated total species richness it is especially low for the site Mühlsee were the extrapolated value for 18 survey does not exceed 75%. This is the only site were the number of surveys necessary to detect 80% of the species are not within the 18 surveys for which the values were extrapolated. For Bachgang and Scheiberbrücke over 80% of the estimated species richness were reached after 7 surveys, for Lanser See, Schwabeneck, Pflutschwiese, and Butterbichl the number of surveys was 8, 12, 13 and 18 respectively. The extrapolated and interpolated values of species richness along with the corresponding lower and upper boundaries of the 95% confidence interval are summarized in table S2 in the supplement.

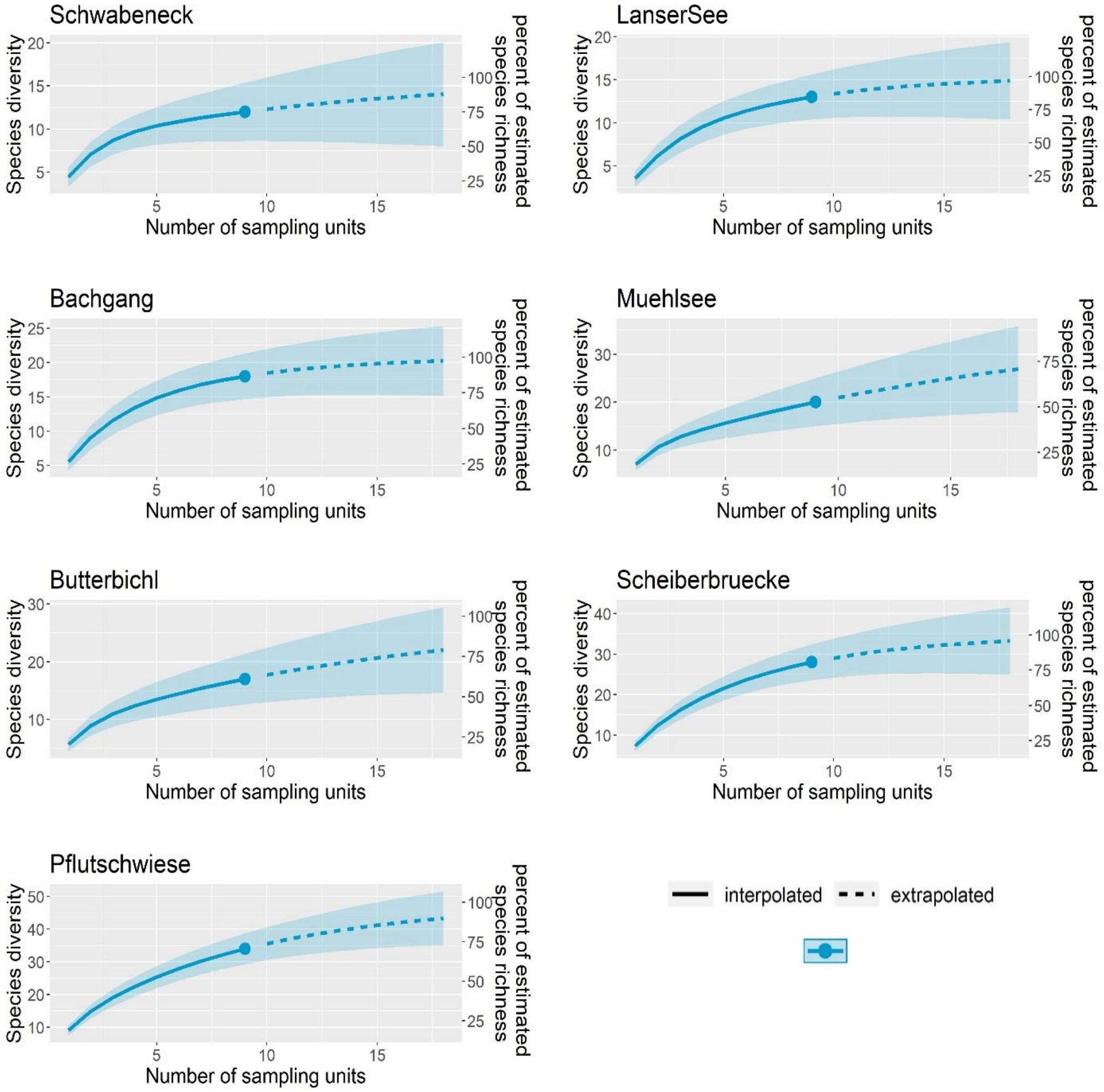

Figure 9 Rarefaction and extrapolation curves for the survey sites with 95% confidence interval. The corresponding values for species richness and the limits of the confidence interval are in the supplements in table S2.

3.2 Information gain of additional surveys

The number of newly detected species with each new survey decreases as the number of surveys increases. The mean number of species that are detected with one survey is 6.10. The mean number of new species that were detected in a second survey in addition to those already recorded at the first one is 3.8. The number of additionally detected species fall below 2 for the fourth survey and below 1 for the ninth survey (fig. 11).

On average 22.3% of the overall species richness were detected with the first survey. The information gain with each additional survey decreases rapidly in the beginning. The mean value is below 15% for the second survey, below 10% for the third and falls below 5% for the sixth survey (fig 11).

The standard deviation is lower for the information gain calculated as percentage of overall species richness.

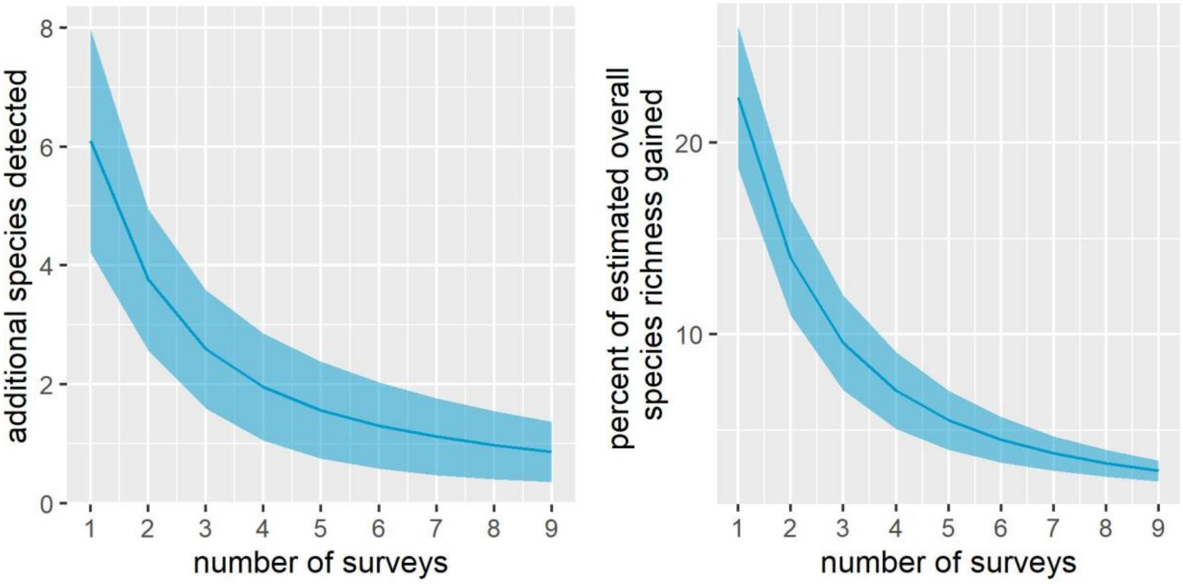

Figure 10 Information gain with additional surveys. Left: mean and standard deviation of new species detected with additional surveys. Right: The mean percent and standard deviation of the overall species richness that is gained with additional surveys.

3.3 Compositional dissimilarity

The relationship between the compositional dissimilarity of two surveys and the time span in between them differed for the different sites. While for all sites the dissimilarity increased with an increasing number of days in between surveys at the beginning, the pattern changed for larger time spans. For the sites Bachgang, Scheiberbrücke and Pflutschwiese the surveys became more similar again when more than about 50 days where in between them. In these cases, a polynomial of degree two was the best fit to the data. For the other survey sites, the best fit of the data was either a logarithmic or reciprocal function indicating an asymptotic relationship. While for the sites Schwabeneck, Lanser See and Butterbichl the dissimilarity index is close to one for the largest time spans it approaches value between 0.6 and 0.7 for the site Mühlsee. For this site the highest values meaning that species differ much in composition were calculated for surveys that were 20 to 50 days apart from each other but this pattern is not reflected well by the function that was the best fit to the data. For the combination of the data from all sites a polynomial model of degree two was the best fit. Dissimilarity increases at the beginning and decreases for surveys that are separated by larger time spans (fig. 12).

In each case the model with the highest adjusted R^2 and the lowest AIC value were chosen. Each model was significant at a level of at least 0.05. The variance explained by the models ranged from 0.187 to 0.650 (table 3).

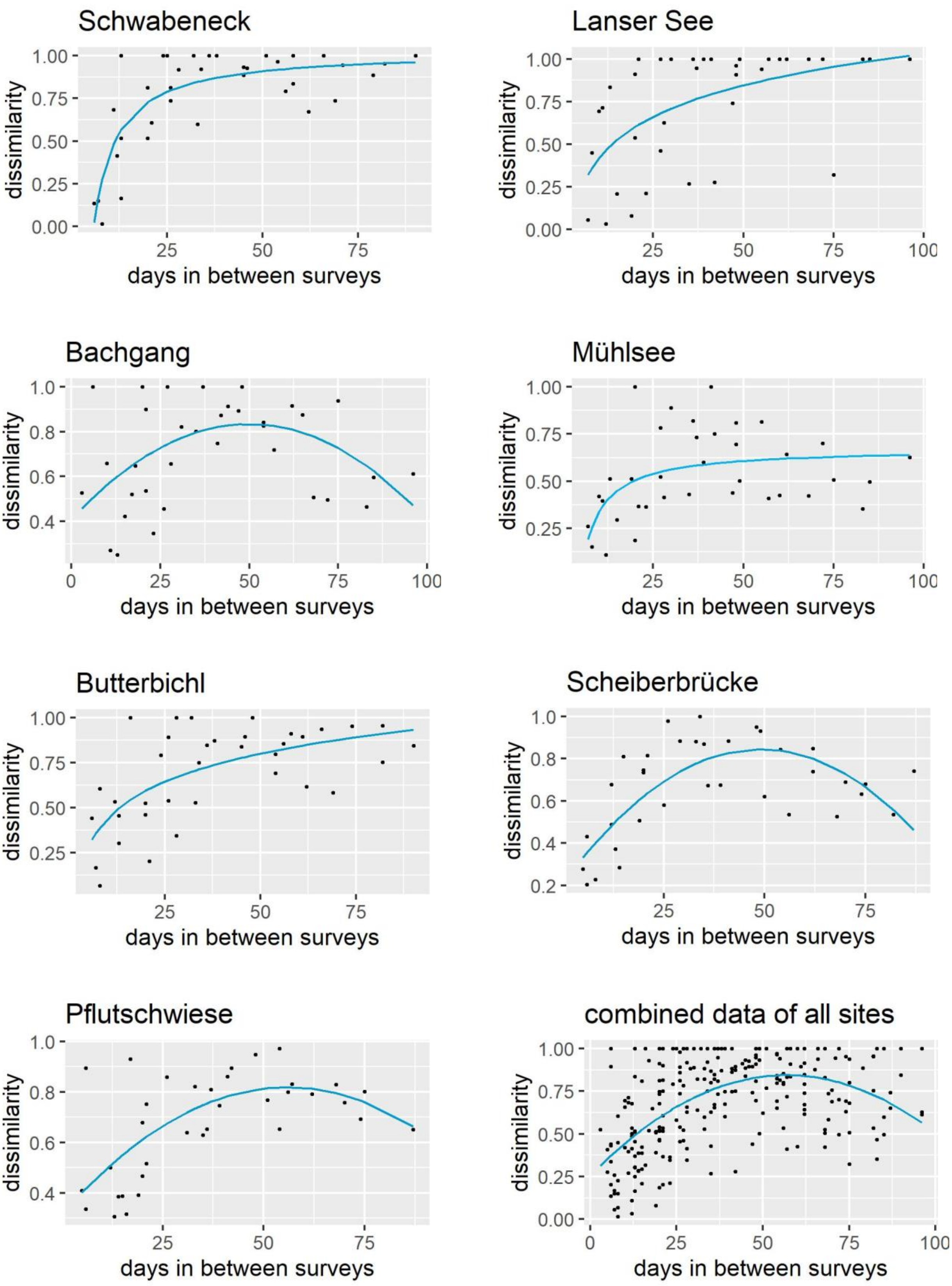

Figure 11 Dissimilarity of surveys against the days in between them and the functional relationship between them: a polynomial of degree 2 for Bachgang, Scheiberbrücke and Pflutschwiese reciprocal for Schwabeneck and Mühlsee-Wiese and logarithmic for Lanser See and Butterbichl. The best fit for the combined data from all sites was a polynomial of degree two. The model parameters are summarized in table S3 in the supplements.

Table 3 functional relationship, adjusted R^2 value and p value of the models that best fitted the relationship between the dissimilarity index and the time between surveys

SITE	RELATIONSHIP	ADJUSTED R^2	P-VALUE
SCHWABENECK	reciprocal	0.650	1.80E-09
LANSER SEE	logarithmic	0.291	4.09E-04
BACHGANG	polynomial of degree 2	0.187	1.25E-02
BUTTERBICHL	logarithmic	0.409	1.62E-05
MÜHLSEE	reciprocal	0.215	2.56E-03
SCHEIBERBRÜCKE	polynomial of degree 2	0.477	8.53E-06
PFLUTSCHWIESE	polynomial of degree 2	0.385	1.25E-04
COMBINATION	Polynomial of degree 2	0.312	2.2E-16

3.4 Temporal distribution of surveys

The rarefied species richness that takes all possible combinations of surveys into account was compared to the species richness that is observed when the combination of surveys is restricted by defining a minimum timespan in between surveys. There was no significant difference between the two approaches (fig. 13).

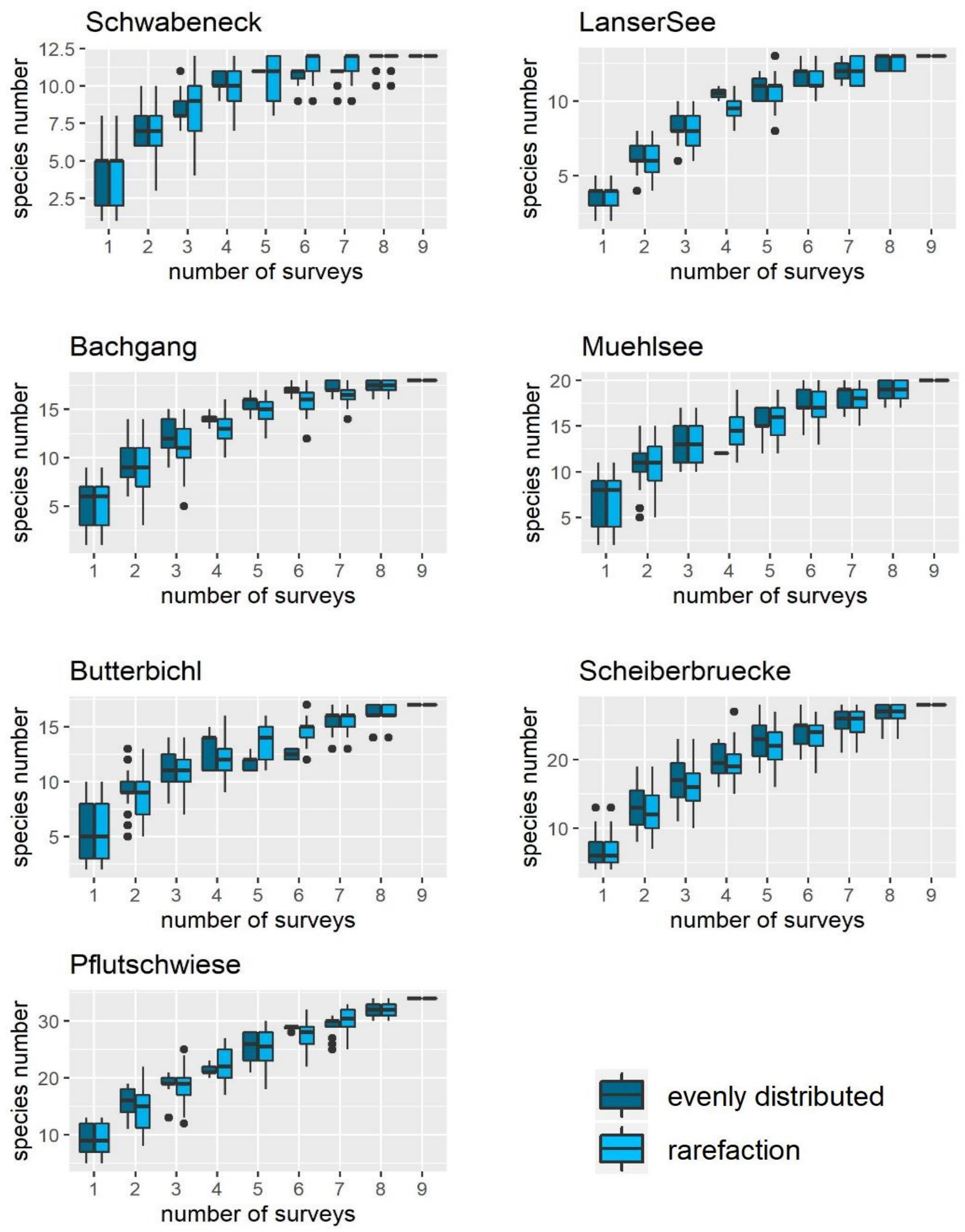

Figure 12 Number of detected species obtained with rarefaction and with an even distribution of the species

3.5 Influence of abundance and vegetation height

A significant influence of vegetation height on species richness could neither be found for any of the seven survey sites nor for the standardized combined data from all sites (table 4 and fig. 14).

Table 4 slope, p value and adjusted R^2 od linear models with species richness as respondent and vegetation height as explanatory variable for the individual survey sites and the combined and standardized data from all sites.

	SLOPE	P.VALUE	R^2
SCHWABENECK	0.137	0.081	0.282
LANSERSEE	- 1.01	0.719	-0.120
BACHGANG	- 0.043	0.616	-0.010
BUTTERBICHL	0.116	0.218	0.094
MUEHLSEE	0.164	0.093	0.258
SCHEIBERBRUECKE	0.053	0.378	-0.015
PFLUTSCHWIESE	0.120	0.104	0.237
COMBINED	0.310	0.020	0.071

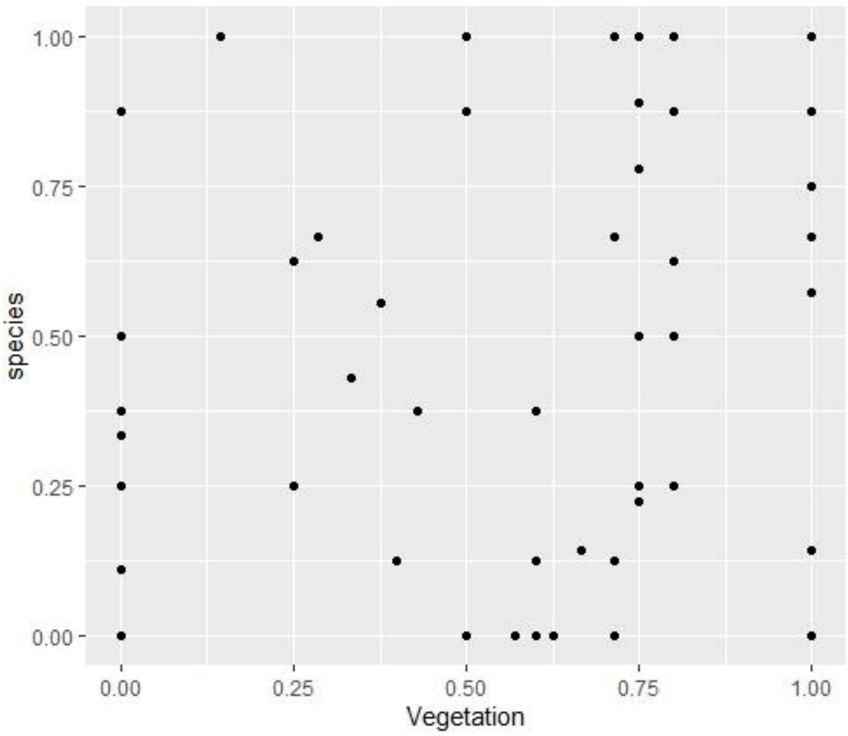

Figure 13 species richness plotted against vegetation height at a survey – species richness and vegetation height were standardized using min-max normalization.

There was a significant relationship between butterfly abundance and number of species during a survey in all but one case (Lanser See). The variance explained with a linear model for those sites where a significant relationship was found ranged from 0.415 to 0.961. For the combined data the relationship was significant and 57.4 % of the variance in species richness could be explained with butterfly abundance (table 5 and fig. 15).

Table 5 slope, p value and adjusted R^2 od linear models with species richness as respondent and abundance as explanatory variable for the individual survey sites and the standardized and combined data from all sites.

	SLOPE	P.VALUE	R^2
SCHWABENECK	0.093	0.036	0.415
LANSERSEE	0.111	0.076	0.295
BACHGANG	0.320	2.24E-06	0.961
BUTTERBICHL	0.235	6.22E-04	0.807
MUEHLSEE	0.276	1.76E-04	0.864
SCHEIBERBRUECKE	0.299	1.47E-05	0.933
PFLUTSCHWIESE	0.230	3.22E-03	0.695
COMBINED	0.810	2E-16	0.697

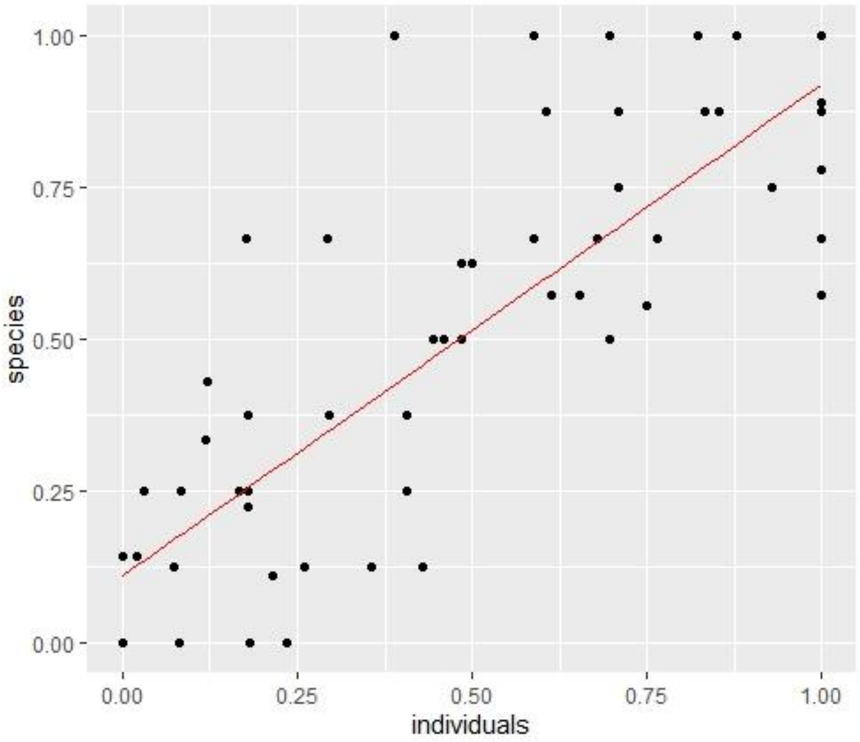

Figure 14 linear model for the relationship between species richness and butterfly abundance during a survey – both parameters were standardized using min-max normalization.

4 Discussion

4.1 Optimize sampling effort

In this study I used the percentage of estimated overall species richness and the information gain attained with additional surveys to develop recommendation for a resource efficient sampling scheme. It was considered desirable to detect 80% of the species that are present on the site over the season. The estimate of overall species richness on the site have a wide 95% confidence interval for most sites. This indicates that more surveys than conducted in this study might be needed for a good estimator of overall species richness. Another reason for the high uncertainty might be that the assumption of rarefaction that samples should be taken from a closed community (Gotelli and Colwell 2011) is not met in this case. There are species that fly in and out of the survey site from the surrounding landscapes and community composition changes in time (Fillecia et al. 2015; Stewart et al. 2020). Especially the higher limit of the confidence interval is often not realistic in comparison to the overall number of species in Tyrol: the upper limit of the interval for the site Mühlsee are 112 species. This is an extremely high number for a single site as there are about 170 butterfly species in Tyrol (Huemer 2013) that are distributed over the diverse landscapes reaching from low valleys to mountain tops. Dennis et al. (1999) observed an increase of detected species even after 18 visits and relate this ongoing accumulation to observations of vagrants – species that don't have their habitat in the surveys site. The estimated overall species richness should therefore be considered carefully and might overestimate the number of species that normally reside on a survey site.

The number of surveys needed to detect 80% of the species differs a lot between the sites and ranges from seven to more than the eighteen surveys to which the data was extrapolated. There is no apparent connection between the species richness and the number of surveys needed to reach 80%. The two sites with the highest number of necessary surveys – Butterbichl and Mühlsee – show intermediate species richness compared to the other sites. Neither do they show a high ratio of species compared to overall abundance which could be an indicator that more effort is necessary to detect many species. The average number of surveys that would be necessary to detect 80% of the species is higher than twelve (it cannot be given precisely as the number for the site Mühlsee is not known) and therefore much higher than the four surveys that are conducted in the Viel-Falter project per site and season. Wikström et a. (2009) found that on average five visits were enough to detect more than 80% of overall species richness. Similar to these results Hardersen and Corezzola (2014) found that 4-5 visits were needed to obtain 75% of the overall species richness for the lowland sites in their study. But they also found that this degree of accuracy was not reached after nine visits for the mountain sites. These differences show, that care has to be taken when comparing results from different study areas obtained with different monitoring designs. In this study 80% of the estimated overall species richness was chosen in order to ensure a good detection of rare species. This is rather a high proportion of overall species richness compared to the two-thirds of estimated total richness that are considered acceptable by Jonason et al. (2010).

Another important aspect when determining the ideal number of surveys per site and season is the information gain that is achieved with each additional survey. In this study it was calculated in the number of new species that was additionally detected with each new survey and in the percent of estimated overall species richness that was added to the detected species with each new survey. As could be expected the standard deviation was higher for the calculation that took absolute species richness into account as the sites differed quite substantially in the estimated overall species richness. The standard deviation is therefore much lower for the number of additionally detected species relative to the overall species richness of the sites. After the fifth survey less than five percent of the overall species richness are added with each new survey. At this point it might be more efficient to use the available resources for additional survey sites rather than for a higher number of surveys per sites. These observations are similar to those made in other studies where the additionally gained information was found to be rather small after five or six surveys (Jonason et al. 2010; Wikström et al. 2009).

Both, proportion of overall species richness and the information gain with additional surveys should be regarded to optimize the survey effort per site. While it is important to get a good picture of the species that reside on a survey site it can be inefficient to execute a lot of surveys on a site adding only few information with each survey. When both aspects are taken into consideration than executing five surveys during the season might be a good recommendation as during these surveys the most abundant species are detected as well as many of the rarer ones. The mean percentage of the estimated overall species richness that is detected after these five surveys is 58.50% (standard deviation: 11.28). This allows to survey more than double the number of sites that would be possible with the same resources for twelve surveys as are needed in average to detect 80% of the estimated overall species richness. After the four surveys that are conducted in the Viel-Falter project there are still more than half of the estimated overall species richness detected (mean: 52.97, standard deviation: 11.19). Four surveys per season would allow to have three times as many survey sites compared to a scheme that is based on the mean number of surveys needed to obtain 80% of the estimated overall species richness.

4.2 Detection of rare species

One reason to aim for the detection of a rather high percentage of overall species richness was to ensure a good detection of rare species. Species can be rare on a single survey site for two reasons. They can usually reside on a site but do so in low abundances or only during a short time of the season.

Another reason for butterflies being only rarely detected on a site might be vagrants – butterflies that are found outside their normal habitat which is situated in the surrounding landscape (Cook et al. 2001). While it is important to detect species that fall into the first category trying to detect each species that normally resides in the surrounding landscape and only seldomly occurs on the survey site would need a lot of resources that can be used better with adding survey sites to the program. The more survey sites there are in a monitoring program the more different kinds of habitats can be covered. Many species have special requirements regarding environmental conditions and biotic interactions especially in their larval stages and are rare because their habitat diminishes (Thomas 2016). The surveying of many different sites makes it more likely to survey suitable habitats of many different species and can allow a better detection of rare species. Therefore, a reduced scheme with five surveys per season as proposed here might also benefit the monitoring of species that are rare and threatened in the study area.

4.3 Temporal distribution of surveys

The rarefaction method assumes that samples are taken independently from each other. This assumption is not strictly fulfilled in this study. While some surveys were conducted only a few days after the one before those at the beginning and end of the season are about three months apart from each other. The species composition of butterflies on a site change during the season (Fillecia et al. 2015; Stewart et al. 2020). Therefore, the number of species that are shared by two surveys also depend on the timespan in between these surveys. In the Viel-Falter program the four surveys that are conducted during the season are supposed to be at least three weeks apart from each other to get a coverage of different times of the season. In this study community similarity decreased for all sites with an increasingly long timespan in between the surveys for timespan of up to 50 days. Afterwards the trend increased while the curves flattened or it was reversed and community similarity increased again for surveys further apart from each other. These results indicate that on some site species that appear in the beginning of the season reoccur later on resulting in a similar species composition in early and late summer. The three sites that show an increase in similarity for longer timespans in between surveys also show a similar pattern in butterfly abundance over the season with two peaks, one in early and one in late summer. This indicates that there were many species with two generations on these sites which cause species richness to be similar at the beginning and the end of the season. Hardersen and Corezzola (2014) found an asymptotic relationship between the number of days in between surveys and compositional similarity for those sites in their study that have a similar elevation range compared to those in this study. The decrease in similarity that was found in this study for surveys with many days in between them did not appear in their data.

The similarity of species composition on survey days close to each other that was observed for all sites could influence the reliability of the rarefaction analysis. In monitoring programs this effect is taken into consideration by applying rules regarding the time in between consecutive surveys while the rarefaction analysis takes all possible combinations of surveys into account. Therefore, combinations of surveys are incorporated into the analysis that would not be made in the Viel-Falter program. Hardersen and Corezzola (2014) found that rarefaction slightly underestimates the number of species that are detected after a certain number of surveys in comparison to an analysis that takes restrictions into account as they are applied in monitoring programs. In this study no significant difference could be found in between the results of the rarefaction analysis and the results from an analysis with a defined minimum timespan in between the surveys. The effect of such restrictions on the number of detected species could be too small to be detected with the analysis used or it could be masked by other factors with a stronger influence. One such factor is the butterfly abundance at the time when the survey is conducted. In this study this relationship was significant for the pooled data from all survey sites and for all but one individual survey site. The average standardized butterfly abundance in this study shows a pattern with two peaks: one in mid-June and one in mid-August. The peak in late summer is slightly higher than that in early summer. Roy et al (2007) showed that many univoltine species also appear in high abundances during the second peak in abundance that is typical for bivoltine species. The study focuses on the most abundant species and they recommend to conduct three surveys per site and season and to focus surveys on late summer to achieve a high efficiency. There are only few species that occur only in spring and early summer such as *A. cardamines* that might be missed with such a scheme. The significant relationship between abundance and detected species and the higher peak in abundance in late summer found in this study supports the approach of conducting more surveys later in the season. However, the summer 2019 was comparably dry and hot in the mountain regions of Austria (ZAMG 2019) which might have caused the second peak to be higher than it would be in years with less favorable weather conditions. Studies that cover more years would be necessary to get a better estimate of seasonal patterns in abundance. With four (as in the Viel-Falter program) or five (as recommended here) surveys it is possible to cover the whole season and have at least one survey in early summer while still being able to focus on late summer and conduct many surveys during a time when abundance is highest and surveys are most efficient.

Vegetation height is another factor that was shown to have an influence on seasonal changes in abundance (Bruppacher et al. 2016) as well as on overall abundance on a site (Milberg et al. 2016). Hence, one could expect similar effects on species richness. In this study no significant effect of vegetation height on butterfly species richness could be found for the individual sites nor for the standardized and pooled data from all sites. Standardizing the data made sure to detect effects of vegetation height on seasonal changes in butterfly abundance which are not to be confounded with the effect of the average vegetation height on overall butterfly abundance on a site. The vegetation height was only documented for the survey site itself and not for the surrounding landscape which could be the reason why there was no significant effect as the surrounding landscape can affect the species richness of a site (Bergman et al. 2004). If surrounding areas are rich in structure and still contain sites with high vegetation butterflies from the surroundings might also be detected on a freshly mown site and species richness can still be high even though there are not many species that stay on the survey site long. Hence, the effect of vegetation height measured only on the survey site might not be very strong and could be masked by other factors such as butterfly phenology.

4.4 Transfer to other surveys sites of the program

The Viel-Falter project covers regions that are characterized by a highly divers landscape and a wide elevational range. The sites studied here lie between 659 and 1123 m a.s.l. while the Viel-Falter program includes sites that are situated as high as 2200 m. The elevation has a strong influence on seasonality and species composition of butterfly communities and the necessary effort to detect a certain percentage of a site's species richness can vary substantially depending on the elevation of survey sites (Hardersen and Corezzola 2014). Therefore, care should be taken when transferring the results of this study to sites on higher elevations. The information gained from surveys by volunteers could help to find the best survey time for sites that exceed the elevation range of this study. These surveys are based on a simplified assessment scheme and are limited to a five minute transect count. While not being able to give information about the times of highest species richness these counts can be used to assess the time of highest butterfly abundance when surveying is most efficient.

4.5 Reference sites

The comparison of survey data from different sites or from different years can be difficult in reduced effort schemes such as the one proposed here. Surveys do not cover the whole season in the detail that is necessary to get a complete picture of seasonal changes in abundance and species richness. The comparison of surveys taken at different times of the season or during different years that vary in weather conditions and seasonal patterns of phenology can therefore be challenging. A possible approach to deal with this difficulty is the establishment of reference sites that are surveyed more often than the other sites and can give information about seasonal changes in abundance. These reference sites can then be used to interpolate the data from the sites with only a few surveys per season and for the calculation of indices for overall abundance as well as for species that occur often enough to obtain reasonable results. The reference sites should be set up at different elevations to account for the effect of elevation on butterfly abundance and phenology. This way these sites would also allow to further optimize surveying in mountain areas and help solving the problem of transferring the data from this study to other sites with different conditions as mentioned above.

5 Conclusion

Based on the results of this study conducting five surveys per site and season seems a reasonable recommendation for those sites of the Viel-Falter program that do not exceed the elevation range of the sites used here. At this number an acceptable amount of species is detected without spending too much resources on surveys that promise only little additional information. The surveys should be focused on late July and August when overall butterfly abundance is highest and monitoring is most efficient. At least one survey should be in early summer though, in order to detect species that do not occur later in the season. In addition, reference sites could give valuable information about seasonal patterns in butterfly abundance under different conditions and allow to use the data collected at the regular sites more efficiently.

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Supplement

Table S1 species lists of the survey sites with the number of observed individuals per species. Species names as used in Stettmer et al. (2007)

Butterbichl

Minois dryas (SCOPOLI, 1763)	40
Maniola jurtina (LINNAEUS, 1758)	22
Polyommatus icarus (ROTTEMBURG, 1775)	20
Vanessa cardui (LINNAEUS, 1758)	15
Coenonympha pamphilus (LINNAEUS, 1758)	13
Pieris rapae (LINNAEUS, 1758)	12
Aphantopus hyperantus (LINNAEUS, 1758)	10
Pieris napi (LINNAEUS, 1758)/ *Pieris bryoniae* (HÜBNER, [1806])	7
Aglais urticae (LINNAEUS, 1758)	5
Colias croceus (GEOFFROY in FOURCROY, 1785)	4
Hesperia comma (LINNAEUS, 1758)	2
Anthocharis cardamines (LINNAEUS, 1758)	1
Colias hyale (LINNAEUS, 1758)/ *Colias alfacariensis* RIBBE, 1905	1
Lycaena phlaeas (LINNAEUS, [1760])	1
Ochlodes sylvanus (ESPER, [1777])	1
Papilio machaon LINNAEUS, 1758	1
Lysandra bellargus (ROTTEMBURG, 1775)	1

Schwabeneck

Minois dryas (SCOPOLI, 1763)	67
Maniola jurtina (LINNAEUS, 1758)	47
Coenonympha pamphilus (LINNAEUS, 1758)	18
Aphantopus hyperantus (LINNAEUS, 1758)	17
Pieris rapae (LINNAEUS, 1758)	16
Vanessa cardui (LINNAEUS, 1758)	12
Pieris napi (LINNAEUS, 1758)/ *Pieris bryoniae* (HÜBNER, [1806])	10
Polyommatus icarus (ROTTEMBURG, 1775)	6

Ochlodes sylvanus (ESPER, [1777])	5
Thymelicus sylvestris (PODA, 1761)	2
Gonepteryx rhamni (LINNAEUS, 1758)	1
Leptidea sinapis (LINNAEUS, 1758)/ *Leptidea reali* REISSINGER, 1990/ *Leptidea juvernica* WILLIAMS, 1946	1

Bachgang

Polyommatus icarus (ROTTEMBURG, 1775)	25
Papilio machaon LINNAEUS, 1758	17
Coenonympha glycerion (BORKHAUSEN, 1788)	15
Coenonympha pamphilus (LINNAEUS, 1758)	9
Erebia medusa ([DENIS & SCHIFFERMÜLLER], 1775)	9
Hesperia comma (LINNAEUS, 1758)	9
Pieris rapae (LINNAEUS, 1758)	9
Maniola jurtina (LINNAEUS, 1758)	7
Colias hyale (LINNAEUS, 1758) /Colias alfacariensis RIBBE, 1905	6
Colias croceus (GEOFFROY in FOURCROY, 1785)	5
Cupido minimus (FUESSLIN, 1775)	3
Vanessa cardui (LINNAEUS, 1758)	3
Aglais urticae (LINNAEUS, 1758)	2
Pieris napi (LINNAEUS, 1758)/ *Pieris bryoniae* (HÜBNER, [1806])	2
Aporia crataegi (LINNAEUS, 1758)	1
Speyeria aglaja (LINNAEUS, 1758)	1
Issoria lathonia (LINNAEUS, 1758)	1
Pyrgus alveus (HÜBNER, [1803])	1

Lanser See

Maniola jurtina (LINNAEUS, 1758)	34
Colias croceus (GEOFFROY in FOURCROY, 1785)	7
Colias hyale (LINNAEUS, 1758)/ *Colias alfacariensis* RIBBE, 1905	7
Pieris rapae (LINNAEUS, 1758)	7

Vanessa cardui (LINNAEUS, 1758)	7
Polyommatus icarus (ROTTEMBURG, 1775)	6
Aglais urticae (LINNAEUS, 1758)	4
Coenonympha pamphilus (LINNAEUS, 1758)	3
Aphantopus hyperantus (LINNAEUS, 1758)	1
Hesperia comma (LINNAEUS, 1758)	1
Issoria lathonia (LINNAEUS, 1758)	1
Papilio machaon LINNAEUS, 1758	1
Pieris brassicae (LINNAEUS, 1758)	1

Mühlsee

Coenonympha pamphilus (LINNAEUS, 1758)	31
Maniola jurtina (LINNAEUS, 1758)	31
Polyommatus icarus (ROTTEMBURG, 1775)	29
Pieris rapae (LINNAEUS, 1758)	21
Colias croceus (GEOFFROY in FOURCROY, 1785)	16
Vanessa cardui (LINNAEUS, 1758)	11
Colias hyale (LINNAEUS, 1758)/ *Colias alfacariensis* RIBBE, 1905	9
Issoria lathonia (LINNAEUS, 1758)	9
Lycaena phlaeas (LINNAEUS, [1760])	7
Anthocharis cardamines (LINNAEUS, 1758)	3
Papilio machaon LINNAEUS, 1758	2
Pieris napi (LINNAEUS, 1758)/ *Pieris bryoniae* (HÜBNER, [1806])	2
Lysandra bellargus (ROTTEMBURG, 1775)	2
Aglais urticae (LINNAEUS, 1758)	1
Gonepteryx rhamni (LINNAEUS, 1758)	1
Hesperia comma (LINNAEUS, 1758)	1
Ochlodes sylvanus (ESPER, [1777])	1
Pyrgus alveus (HÜBNER, [1803])	1
Pyrgus malvoides (ELWES & EDWARDS, 1897)	1
Thymelicus sylvestris (PODA, 1761)	1

Scheiberbrücke

Coenonympha pamphilus (LINNAEUS, 1758)	29
Pieris rapae (LINNAEUS, 1758)	19
Colias croceus (GEOFFROY in FOURCROY, 1785)	9
Polyommatus icarus (ROTTEMBURG, 1775)	9
Erebia ligea (LINNAEUS, 1758)	8
Pieris napi (LINNAEUS, 1758)/ *Pieris bryoniae* (HÜBNER, [1806])	8
Vanessa cardui (LINNAEUS, 1758)	7
Maniola jurtina (LINNAEUS, 1758)	6
Aglais urticae (LINNAEUS, 1758)	5
Aphantopus hyperantus (LINNAEUS, 1758)	5
Cyaniris semiargus (ROTTEMBURG, 1775)	5
Anthocharis cardamines (LINNAEUS, 1758)	4
Brenthis ino (ROTTEMBURG, 1775)	4
Erebia medusa ([DENIS & SCHIFFERMÜLLER], 1775)	4
Melitaea athalia (ROTTEMBURG, 1775)	4
Speyeria aglaja (LINNAEUS, 1758)	3
Argynnis paphia (LINNAEUS, 1758)	2
Gonepteryx rhamni (LINNAEUS, 1758)	2
Leptidea sinapis (LINNAEUS, 1758)/ *Leptidea juvernica* WILLIAMS, 1946	2
Lycaena tityrus (PODA, 1761)	2
Thymelicus sylvestris (PODA, 1761)	2
Vanessa atalanta (LINNAEUS, 1758)	2
Aporia crataegi (LINNAEUS, 1758)	1
Aricia Artaxerxes (FABRICIUS, 1793), *Aricia agestis* ([DENIS & SCHIFFERMÜLLER], 1775)	1
Aglais io (LINNAEUS, 1758)	1
Lycaena phlaeas (LINNAEUS, [1760])	1
Papilio machaon LINNAEUS, 1758	1
Pieris brassicae (LINNAEUS, 1758)	1

Pflutschwiese

Maniola jurtina (LINNAEUS, 1758)	31

Coenonympha pamphilus (LINNAEUS, 1758)	28
Erebia aethiops (ESPER, [1777])	18
Thymelicus sylvestris (PODA, 1761)	18
Erebia medusa ([DENIS & SCHIFFERMÜLLER], 1775)	15
Polyommatus icarus (ROTTEMBURG, 1775)	12
Pieris rapae (LINNAEUS, 1758)	11
Vanessa cardui (LINNAEUS, 1758)	11
Erebia alberganus (DE PRUNNER, 1798)	9
Melitaea athalia (ROTTEMBURG, 1775)	7
Aphantopus hyperantus (LINNAEUS, 1758)	4
Aricia artaxerxes (FABRICIUS, 1793)	4
Leptidea sinapis (LINNAEUS, 1758)/*Leptidea juvernica* WILLIAMS, 1946	4
Lycaena phlaeas (LINNAEUS, [1760])	4
Lycaena tityrus (PODA, 1761)	4
Ochlodes sylvanus (ESPER, [1777])	4
Aglais urticae (LINNAEUS, 1758)	3
Colias croceus (GEOFFROY in FOURCROY, 1785)	3
Colias hyale (LINNAEUS, 1758)/ *Colias alfacariensis* RIBBE, 1905	3
Pieris brassicae (LINNAEUS, 1758)	3
Speyeria aglaja (LINNAEUS, 1758)	2
Carterocephalus palaemon (PALLAS, 1771)	2
Coenonympha glycerion (BORKHAUSEN, 1788)	2
Papilio machaon LINNAEUS, 1758	2
Anthocharis cardamines (LINNAEUS, 1758)	1
Aporia crataegi (LINNAEUS, 1758)	1
Boloria euphrosyne (LINNAEUS, 1758)	1
Boloria selene ([DENIS & SCHIFFERMÜLLER], 1775)	1
Pararge aegeria (LINNAEUS, 1758)	1
Pieris napi (LINNAEUS, 1758)/ *Pieris bryoniae* (HÜBNER, [1806])	1
Polygonia c-album (LINNAEUS, 1758)	1
Lysandra bellargus (ROTTEMBURG, 1775)	1
Polyommatus eros (OCHSENHEIMER, 1808)	1
Vanessa atalanta (LINNAEUS, 1758)	1

Table S2 Interpolated and extrapolated values of species richness with upper and lower boundaries of the 95% confidence interval based on the Chao2 estimator.

SCHWABENECK

SURVEYS	METHOD	SPECIES NUMBER	LOWER LIMIT	UPPER LIMIT
1	interpolated	4.444	3.444	5.445
2	interpolated	7.083	5.871	8.296
3	interpolated	8.679	7.33	10.028
4	interpolated	9.69	8.145	11.236
5	interpolated	10.381	8.598	12.164
6	interpolated	10.893	8.857	12.928
7	interpolated	11.306	9.015	13.596
8	interpolated	11.667	9.123	14.211
9	observed	12	9.21	14.79
10	extrapolated	12.308	9.264	15.352
11	extrapolated	12.592	9.299	15.885
12	extrapolated	12.854	9.318	16.39
13	extrapolated	13.096	9.324	16.868
14	extrapolated	13.319	9.318	17.32
15	extrapolated	13.526	9.303	17.748
16	extrapolated	13.716	9.28	18.151
17	extrapolated	13.892	9.251	18.533
18	extrapolated	14.054	9.215	18.892

LANSER SEE

SURVEYS	METHOD	SPECIES NUMBER	LOWER LIMIT	UPPER LIMIT
1	interpolated	3.556	2.65	4.461
2	interpolated	6.167	4.833	7.501
3	interpolated	8.083	6.499	9.668
4	interpolated	9.5	7.744	11.256
5	interpolated	10.563	8.67	12.457
6	interpolated	11.381	9.357	13.405
7	interpolated	12.028	9.864	14.191

8	interpolated	12.556	10.235	14.877
9	observed	13	10.501	15.499
10	extrapolated	13.374	10.677	16.071
11	extrapolated	13.689	10.78	16.599
12	extrapolated	13.955	10.823	17.087
13	extrapolated	14.178	10.82	17.537
14	extrapolated	14.367	10.781	17.952
15	extrapolated	14.525	10.715	18.335
16	extrapolated	14.659	10.629	18.688
17	extrapolated	14.771	10.529	19.013
18	extrapolated	14.866	10.419	19.313

BACHGANG

SURVEYS	METHOD	SPECIES NUMBER	LOWER LIMIT	UPPER LIMIT
1	interpolated	5.556	4.339	6.772
2	interpolated	9.056	7.298	10.813
3	interpolated	11.56	9.445	13.674
4	interpolated	13.437	11.058	15.815
5	interpolated	14.857	12.267	17.447
6	interpolated	15.94	13.17	18.711
7	interpolated	16.778	13.846	19.71
8	interpolated	17.444	14.357	20.532
9	observed	18	14.753	21.247
10	extrapolated	18.463	15.045	21.881
11	extrapolated	18.849	15.247	22.451
12	extrapolated	19.17	15.373	22.967
13	extrapolated	19.438	15.438	23.439
14	extrapolated	19.661	15.452	23.871
15	extrapolated	19.848	15.426	24.269
16	extrapolated	20.003	15.368	24.637
17	extrapolated	20.132	15.287	24.977
18	extrapolated	20.239	15.186	25.293

BUTTERBICHL

SURVEYS	METHOD	SPECIES NUMBER	LOWER LIMIT	UPPER LIMIT
1	interpolated	5.667	4.713	6.62
2	interpolated	8.917	7.55	10.283
3	interpolated	10.929	9.042	12.815
4	interpolated	12.349	9.922	14.776
5	interpolated	13.492	10.556	16.428
6	interpolated	14.488	11.091	17.885
7	interpolated	15.389	11.583	19.195
8	interpolated	16.222	12.057	20.387
9	observed	17	12.523	21.477
10	extrapolated	17.726	12.958	22.493
11	extrapolated	18.403	13.359	23.448
12	extrapolated	19.036	13.722	24.35
13	extrapolated	19.626	14.047	25.205
14	extrapolated	20.177	14.335	26.018
15	extrapolated	20.691	14.588	26.794
16	extrapolated	21.171	14.807	27.535
17	extrapolated	21.619	14.995	28.242
18	extrapolated	22.037	15.155	28.919

MÜHLSEE

SURVEYS	METHOD	SPECIES NUMBER	LOWER LIMIT	UPPER LIMIT
1	interpolated	7	5.878	8.122
2	interpolated	10.611	9.208	12.014
3	interpolated	12.798	11.118	14.477
4	interpolated	14.365	12.347	16.383
5	interpolated	15.659	13.277	18.04
6	interpolated	16.833	14.088	19.579
7	interpolated	17.944	14.84	21.049
8	interpolated	19	15.535	22.465

9	observed	20	16.164	23.836
10	extrapolated	20.947	16.715	25.179
11	extrapolated	21.845	17.192	26.498
12	extrapolated	22.695	17.599	27.791
13	extrapolated	23.501	17.944	29.058
14	extrapolated	24.264	18.23	30.297
15	extrapolated	24.987	18.465	31.508
16	extrapolated	25.672	18.654	32.689
17	extrapolated	26.321	18.802	33.839
18	extrapolated	26.935	18.913	34.958

SCHEIBERBRÜCKE

SURVEYS	**METHOD**	**SPECIES NUMBER**	**LOWER LIMIT**	**UPPER LIMIT**
1	interpolated	7.333	6.048	8.619
2	interpolated	12.361	10.377	14.346
3	interpolated	16.119	13.625	18.613
4	interpolated	19.111	16.207	22.015
5	interpolated	21.571	18.324	24.819
6	interpolated	23.619	20.068	27.17
7	interpolated	25.333	21.499	29.168
8	interpolated	26.778	22.665	30.89
9	observed	28	23.603	32.397
10	extrapolated	29.034	24.338	33.731
11	extrapolated	29.909	24.896	34.922
12	extrapolated	30.65	25.307	35.992
13	extrapolated	31.276	25.596	36.956
14	extrapolated	31.806	25.786	37.827
15	extrapolated	32.255	25.895	38.615
16	extrapolated	32.635	25.94	39.329
17	extrapolated	32.956	25.934	39.978
18	extrapolated	33.228	25.887	40.568

PFLUTSCHWIESE

SURVEYS	METHOD	SPECIES NUMBER	LOWER LIMIT	UPPER LIMIT
1	interpolated	9.111	7.857	10.366
2	interpolated	14.833	12.877	16.79
3	interpolated	19.048	16.52	21.576
4	interpolated	22.476	19.451	25.501
5	interpolated	25.405	21.934	28.876
6	interpolated	27.964	24.082	31.847
7	interpolated	30.222	25.946	34.498
8	interpolated	32.222	27.55	36.894
9	observed	34	28.91	39.09
10	extrapolated	35.58	30.041	41.119
11	extrapolated	36.985	30.965	43.004
12	extrapolated	38.234	31.705	44.762
13	extrapolated	39.343	32.285	46.402
14	extrapolated	40.33	32.726	47.934
15	extrapolated	41.207	33.049	49.365
16	extrapolated	41.986	33.271	50.701
17	extrapolated	42.679	33.41	51.949
18	extrapolated	43.295	33.477	53.113