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Biodiversity conservation and sustainable management in the vineyard agroecosystem:
an integrated approach for different trophic levels

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Abstract

In the general introduction the importance to preserve biodiversity and ecosystem functioning in agroecosystems and the basic ideas which have oriented the present thesis were presented.

Firstly, we presented results of biotic and abiotic factors affecting species composition and their related functional traits (Chapters 2 and 3). In 2009, a pilot study provided useful preliminary insights for the present study with respect: to selection of effective sampling techniques for arthropods, to develop the main study design, to assess statistical analysis techniques, and to develop further research questions and hypotheses. The Auchenorrhyncha (Insecta: Hemiptera: Fulgoromorpha and Cicadomorpha) were used as model taxon. Environmental and management variables accounted for most of the variance in the Auchenorrhyncha (leafhoppers hereafter) assemblages. In particular, pesticide use (insecticide and herbicide) and mowing of embankments were the best management predictors of leafhopper species composition. With increasing management pressure, the number of indicator species and particularly the specialists (i.e. stenotopic and oligotopic species) decreased dramatically. In 2011, an extensive study was carried out to highlight the relative importance of environmental factors and biotic interactions shaping community assemblages at two trophic levels: plants (primary producers) and leafhoppers (phytophagous). The tested models explained more than half of the variation in plant and leafhopper assemblages (51.8% and 54.1%, respectively), and the most important variables were topographic (mainly slope of sampling sites) and biotic ones. Abiotic filtering processes were relatively more important than biotic ones (plants: 9.6% vs 4.9%; leafhoppers: 14.8% vs 3.8%). Species co-occurrence of overall plant and leafhopper communities showed a clear evidence of non-randomness segregated patterns. However, pairwise co-occurrence analyses showed an aggregated pattern for polyphagous and common leafhoppers species (15 species pairs out of 20) and for monophagous leafhoppers and their potential host plants; and a high frequency of segregated species pairs for plant communities (40 out of 57).

Secondly, we produced a list of indicator plant species predictive of high taxonomic and functional biodiversity values. We considered ten widely used functional traits and selected six taxonomic and functional biodiversity indices. We used a two-step multivariate analysis to select 52 species significant indicators for high and mid-to-high biodiversity values. Out of all indicator species, 24 (46%) were exclusively selected by functional biodiversity indices whereas only 10 (19%) were associated with taxonomic indices. Eighteen (35% of the total) species were selected by both types of indices. Our results emphasized the need to consider functional aspects of biodiversity in diversity-conservation strategies when the objectives are to preserve both taxonomic diversity and ecosystem functioning (Chapter 4).

Thirdly, we proposed a conceptual framework as a tool for the selection of suitable indicators to measure botanical quality in the vineyard agroecosystem. This framework was devised based on four criteria for the selection of indicator plant species: 1) Management intensity, 2) Components of biodiversity, 3) Vulnerability and threat of extinction, 4) Real and potential harm to biodiversity. Applying the framework to the vineyards of Southern Switzerland allowed to select a total of 118 species (Chapter 5).

Lastly, we investigated the role of leafhoppers as vectors of two plant pathogens (phytoplasmas) which cause two important diseases to grapevine (Flavescence dorée- FD and Bois noir- BN). The diseases control strategies are not always effective and an in-depth study on the epidemiological cycles of the pathogens at regional scale is of paramount importance. In this study, we investigated the occurrences of known and potential vectors of phytoplasmas in vineyards and the multilocus sequence typing (MLST) approach was used to characterize the genetic diversity of phytoplasma isolates in the insect bodies. Out of 167 leafhopper species recorded, 27 were known or potential vectors of phytoplasmas and five of those tested positive for phytoplasmas. *Scaphoiodeus titanus* was infected by 16SrV-D subgroup phytoplasma and no clear relationship between its population density and disease outbreaks was observed. *Orientus ishidae* harboured 16SrV-C and 16SrV-D subgroups suggesting its potential role in spreading 16SrV-C phytoplasma isolates from arboreal plants to grapevine, and FD-D from grapevine to grapevine. *Hyalesthes obsoletus* was infected by BN phytoplasmas, tuf-types *a* and *b*, however it was collected with relatively low abundance. *Reptalus panzeri* and *R. cuspidatus* tested positive to tuf-type *b*, but only *R. cuspidatus* was common and abundant in the investigated vineyards. To define the range of alternative vectors using a detailed approach on regional scale provides background information to get a more clear vision on the spreading of phytoplasmas in the vineyards (Chapter 6).

We discuss the general consequences of our findings in the frame of sustainable management strategy of vineyards, as well as future lines of research in a concluding chapter.

Key words: arthropods, plants, viticulture, community assembly, phytosanitary issue

Résumé

Dans l'introduction générale, nous précisons l'importance de la conservation de la biodiversité et du fonctionnement des écosystèmes dans les agroécosystèmes ainsi que les réflexions à la base de la présente thèse.

Nous avons tout d’abord présenté les résultats en rapport avec les facteurs biotiques et abiotiques qui affectent la composition des espèces et leurs traits fonctionnels (Chapitres 2 et 3). En 2009, une étude pilote a fourni des indications préliminaires utiles pour la présente étude en ce qui concerne: le choix de techniques d'échantillonnage efficaces pour les arthropodes, l’élaboration de la conception de l'étude principale, l’évaluation des techniques d'analyse statistique ainsi que le développement d'autres questions et hypothèses de recherche. Les Auchenorrhynches (Insecta: Hemiptera: Fulgoromorpha et Cicadomorpha) ont été utilisés comme taxons modèle. Les variables liées à l'environnement et à la gestion expliquent la plupart de la variance dans les communautés des Auchenorrhynches (cicadelles ci-dessous). En particulier, l'utilisation de pesticides (insecticides et herbicides) et la fauche des talus se sont révélés être les meilleurs prédicteurs de la composition des espèces indicatrices des cicadelles : Lorsque la pression de gestion augmente, le nombre des espèces indicatrices, et en particulier les spécialistes (c'est-à-dire les espèces oligotopes (et stenotopes), diminuent dramatiquement. En 2011, une large étude a été conduite afin d’évaluer l’importance relative des facteurs environnementaux et des interactions biotiques qui déterminent les communautés biologiques à deux niveaux trophiques: végétaux (producteurs primaires) et cicadelles (phytophages). Les modèles testés expliquent plus de la moitié de la variation dans les communautés végétales (51.8%) et de cicadelles (54.1%). Les variables les plus importantes ont été de nature topographique (principalement la pente du site d’échantillonnage) et biotique. Les processus abiotiques ont été plus importants que les processus biotiques (végétaux: 9.6% vs 4.9%; cicadelles: 14.8% vs 3.8%). La co-occurrence des espèces de végétaux et de cicadelles a clairement révélé une distribution ségréguée non-aléatoire des communautés. Cependant, l’analyse de co-ocurrence par paires a mis en évidence un distribution agrégée pour les espèces de cicadelles polyphages et communes (15 couples d’espèces sur 20) et pour les cicadelles monophages et leurs plantes hôtes potentielles ainsi qu’une haute fréquence de couples ségrégués pour les végétaux (40 sur 57).

En deuxième lieu, nous avons établi une liste de plantes indicatrices qui prédisent des valeurs plus élevées de la biodiversité taxonomique et fonctionnelle. Nous avons examiné 10 caractères fonctionnels largement utilisés et nous avons sélectionné 6 indices taxonomiques et fonctionnels. Nous avons utilisé une analyse multivariée à deux niveaux pour sélectionner 52 espèces indiquant de façon significative des valeurs élevées ou des valeurs moyennes à élevées de la biodiversité. Parmi les espèces indicatrices, 24 (46%) ont été exclusivement sélectionnées par les indices de biodiversité fonctionnelle et seulement 10 (19%) ont été associées à des indices taxonomiques. Dix-huit espèces (35% du total) ont été sélectionnées à partir des deux types d'indices. Nos résultats soulignent la nécessité de tenir compte des aspects fonctionnels dans les stratégies de conservation de la biodiversité lorsque les objectifs sont de préserver tant la diversité taxonomique que le fonctionnement des écosystèmes (Chapitre 4).

En troisième lieu, nous avons proposé un cadre conceptuel comme outil de sélection d'indicateurs en vue de mesurer la qualité botanique du vignoble. Ce cadre a été élaboré sur la base de quatre critères: 1) intensité de gestion, 2) composants de la biodiversité, 3) vulnérabilité et menace d'extinction, 4) dommages réels et potentiels pour la biodiversité. L'application du cadre sur les vignobles au sud de la Suisse nous a permis de sélectionner un total de 118 espèces (Chapitre 5).

Enfin, nous avons étudié le rôle des cicadelles comme vecteurs de deux agents pathogènes des plantes (phytoplasmes) causant deux maladies importantes de la vigne (flavescence dorée FD et bois noir BN). Comme les stratégies de lutte contre la maladie ne sont pas toujours efficaces, il est essentiel de faire une étude approfondie sur les cycles épidémiologiques des agents pathogènes au niveau régional. Dans cette étude, nous avons étudié l'incidence des vecteurs connus et potentiels de phytoplasmes dans les vignobles par une approche de typage génétique par séquençage multi-locus (MLST) pour caractériser la diversité génétique des isolats de phytoplasme dans le corps des insectes. Sur l'ensemble des 167 espèces de cicadelles identifiés, 27 étaient vectrices connues ou vectrices potentiels de phytoplasmes et cinq d'entre elles se sont révélées être infectées. *Scaphoiodeus titanus* était infecté par le phytoplasme du sous-groupe 16SrV-D et il n'a pas été observé de relation claire entre la densité de la population et l'apparition de la maladie. *Orientus ishidae* était infectée par les sous-groupes 16SrV-C et 16SrV-D, ce qui suggère un rôle potentiel de transfert du phytoplasme 16SrV-C sur la vigne à partir de plantes arborescentes et du 16SrV-D entre plants de vigne. *Hyalesthes obsoletus* était infectée par le phytoplasme BN, tuf-types a et b, mais la collecte était relativement faible. *Reptalus panzeri* et *R. cuspidatus* étaient infectées par type de tuf b, mais seule *R. cuspidatus* était commune et abondante dans les vignobles étudiés. En définissant la gamme des vecteurs alternatifs à l'aide d'une approche détaillée au niveau régional, il est possible d'obtenir des informations de base en vue de mieux comprendre la propagation des phytoplasmes dans les vignobles (Chapitre 6).

Dans le chapitre final, nous discutons les conséquences générales de nos résultats dans le cadre de la stratégie de gestion durable des vignobles, ainsi que les lignes de recherche futures.

Mots-clés: arthropodes, plantes, viticulture, communauté biologique, risque phytosanitaire

Contents

Chapter 1 General Introduction

1.1 Agricultural areas as opportunities for biodiversity and ecosystem conservation

Agro-ecosystems cover nearly one-third of the world's landmass and in the European continent alone about 50% (Millennium Ecosystem Assessment 2005). Hence, the land area is heavily influenced by cropland, planted pastures, and livestock grazing systems. Agriculture provides easily measured services to satisfy the needs of people, such as food, fiber and bio-energy production (FAO 2011), but also a range of other social and environmental outcomes, some positive and some negative (externalities) (Morris & Burgess 2012). Among these, environmental services have received the greatest attention in the last 50 to 60 years, in particular the impacts of negative externalities of agricultural activities, preservation of biodiversity and impacts of positive externalities of agriculture. Agriculture is a part of an ecosystem rather than being external to it. Consequently, all farming activities can change the natural environment in many ways with consequences beyond field margins. Agricultural intensification by use of high-yielding crop varieties, fertilization, irrigation, and pesticides has had well-known negative consequences. These include de-regulation of climate and biogeochemical cycles, depletion of soil fertility, discharge of hazardous substances, disrupting sources of food and shelter for wild biodiversity, but also a fundamental reduction of genetic diversity in agricultural products due to market needs (Matson *et al*. 1997). On the other hand, positive environmental externalities—such as water supply, nutrient fixation, soil formation, maintaining of the farmland biodiversity, flood control, and carbon sequestration—can also be provided by adopting sustainable agriculture practices (Rasul 2009; Bowe & der Horst 2015). For all these reasons, agriculture could pose a threat to wild plants, animal species, natural ecosystem functions, and services upon which both humans and wildlife depend (Gaigher & Samways 2010; Tscharntke *et al*. 2012; Bohan *et al*. 2013). Nevertheless, agriculture presents an opportunity to realize "win-win" situations where biodiversity is supported by sustainable rural development (Poláková *et al*. 2011; Fehér & Beke 2013). Given that demands on global agricultural production are increasing with no counter trend signals, it is imperative to move towards trade-offs between increased productivity and enhance conservation of biodiversity and related ecosystem services. Achieving this milestone, however, requires quantitative knowledge about ecosystem responses to land use (DeFries, Foley & Asner 2004).

In this respect, productive, environmentally friendly, and socially responsible agriculture depends on the integration of ecological, economic, and social points of view. This concept was unified under the term "eco-agriculture" coined in 1970 by Charles Walters in the belief that unless agriculture was ecological it could not be economical (Walters 2003). An agroecosystem cannot have ecological integrity unless it also has social and economic integrity. It cannot be socially just unless it is also ecologically and economically just. Further, it cannot be economically viable unless it is ecologically and socially viable (Kristiansen *et al*. 2006). Promoting eco-agriculture is the main focus for sustainable production which is brought to fruition at two main levels: individual and collective. The first level is related to the choices of each single farmer and his or her capability to use natural resources efficiently, conserving them and ensuring sustainable production. In the second, positive and negative environmental externalities caused by agriculture generate social demands that must be satisfied by action that is collective, and thereby, political (Arzeni, Esposti & Sotte 2001; Paillotin 2013). In this perspective, it is necessary to consider farmland habitats in all of the various biodiversity and ecosystem conservation measures (Kleijn *et al*. 2011). In particular, biodiversity protection regimes throughout the agricultural landscape are important to support the single farmers, the entire agriculture sector and, last but not least, the value of natural areas in the vicinity of farming areas. Given that political structures will need to develop effective biodiversity protection and appreciation measures, and that individuals will be required to accept these responsibilities, it is important that the concept of agricultural biodiversity be clearly defined and understood by all concerned. Although the Convention on Biological Diversity (1992) does not contain a definition of agro-biodiversity, in Decision V/5—adopted during the Fifth Conference of the Parties of CBD (COP-V) in 2000—agricultural biodiversity is defined as “a broad term that includes all components of biological diversity of relevance to food and agriculture, and all components of biological diversity that constitute the agroecosystem: the variety and variability of animals, plants and microorganisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agroecosystem, its structure and processes” (Santilli 2013).

The complexity of the concept itself forces the need for specific action to maintain biodiversity for each of the three levels mentioned in the above definition. In Europe, there are currently no biodiversity conservation policies that are capable of reaching this goal. In particular, ecosystem services collectively represent a component of biodiversity that is still difficult to measure, monetize, and integrate into agroecosystem conservation measures (Kleijn & Sutherland 2003; Power 2010; Batáry *et al*. 2015).

Scientific research on agriculture should provide background knowledge and solutions based on an interdependent process, and provide support towards the formalizing of policies for the protection of biodiversity. On one hand, it should define the potential of biodiversity existing in a given area, the characteristics of biological relationships between habitats, and the resilience of agro-ecosystems to disturbances. On the other hand, it should also implement innovative tools and sustainable solutions to utilize and manage natural resources.

In recent decades, traditional scientific approaches to biological diversity conservation—that are merely based on the protectionist point of view (the intrinsic value of biodiversity)—have gradually shifted towards and integrated the conservation of ecologically important species which play a crucial role in ecosystems, leading to the conservation of ecosystem processes mediated by biological communities (Ehrlich 2002; Turner *et al*. 2007; Goldman *et al*. 2008; Eigenbrod *et al*. 2009).

The relationship between crops and the associated wild environment changes profoundly according to the type of cultivated plant and edaphic conditions, making it impossible to generalize regarding the management systems to adopt. For example, with perennial commercial crops, cover crops (permanent presence of a diversified resident flora) are usually used in association with the cultivated plant representing an opportunity to convert a monoculture into a more biologically diverse agroecosystem. Vineyard are considered one of the most important perennial crops and the management activities can cause considerable environmental impacts and income (Costantini & Barbetti 2008; Lalevic *et al*. 2013; Lieskovský & Kenderessy 2014). For this reason, vineyards can represent a good model for the development and planning of management strategies for the purpose of enhancing biodiversity in the field.

1.2 Wine-growing regions in Switzerland

Switzerland is a small yet highly diverse country, with around 65% of its land area covered by high mountains, and arable land accounting for about 10%. The viticulture area—which covers 15,000 hectares (ha)—represents about 0.3% of the total land area and plays an important role in shaping the geographical and economic landscape. Swiss policy has always paid close attention to the preservation of agricultural heritage, including the conservation of some of the world's most picturesque yet inconvenient vineyards, which are mainly located in the western French part of the country. The most distinctive vineyards are dislocated on terraces and steep slopes, and are usually scattered in small plots (national mean < 1 ha per grower). More than 85% of all vineyards are cultivated in an environmentally-friendly way (Viret 2013).

Wine-growing areas in Switzerland are notably grouped into three main regions based on linguistic boundaries: French-, German-, and Italian-speaking. The first one encompasses mainly the Cantons of Geneva, Vaud, Valais, Freiburg, Neuchâtel, Jura and the Lake of Biel area in the canton of Bern. The second includes, the Cantons of Basel-Country, Basel-City, Solothurn, Aargau, Schaffhausen, Thurgau, St. Gallen, Glarus, Zurich, Schwyz, Zug, Lucerne, Obwalden, Nidwalden, Appenzell Outer Rhodes and Appenzell Inner Rhodes, Uri, the Grisons, and Bern, with the exception of the Lake of Bienne area. The last area is the only Italian-speaking canton of Ticino and a small part of the canton of Grisons. In terms of their extension, the four most important viticultural cantons are: Valais (5,000 ha), Vaud (3,878 ha), Geneva (1,494 ha) and Ticino (1,100 ha).

From a biological point of view, it is more appropriate to use floristically-defined regions (biogeographical regions) as defined by Wohlgemuth (1996)- based on a study by Gonseth *et al.* (2001)- which consider both flora and fauna in the differentiation of regions. Overall, a total of six main regions can be identified. According to this regionalization, it is possible to group the most important wine-growing areas into three out of six biogeographical regions: the Plateau which comprises the cantons of Vaud and Geneva, the Central Western Alps which comprises the cantons of Valais and the South side of the Swiss Alps which consists of the canton of Ticino and a small part of the canton of Grisons. In the present study, we consider this last region as the study area.

1.3 Rationale of the thesis: concept of sustainability and integrated production

In recent years, socio-environmental issues have become an integral part of agricultural policy objectives worldwide. The concept of sustainable agriculture arises from the need to “sustain” the production of food that respects and reflects the needs or priorities of the various components within a community. These include, for example, maintaining high quality standards and authenticity of food and life products, maintaining a viable economy, the improvement of environmental and landscape quality, and the right to benefit from a healthy environment. Given that these legitimate needs must coexist in harmony within a productive framework, it becomes evident that the fundamental key lies in the identification of points of integration that can facilitate these interests. The search for an ideological framework wherein multiple community interests may be integrated is a participative process where all interested parties (social, institutional, and scientific community stakeholders) identify production guidelines, and management solutions and orientations through constant mediation and negotiation.

The foundations of sustainable production, as well as any resultant decisions and actions, require definition at the regional level, since residential communities are in constant evolution and often have varying needs.

At the international level, the notion of production that best represents the principles of sustainability are found in the Integrated Production concept - see Baggiolini (1990) for a review. In Switzerland, the rules of Integrated Production for vineyards were introduced in 1991. However, at the end of the 1970s, Mario Baggiolini promoted and actively supported Integrated Production that he defined as

“a new paradigm in which nature and techniques, biology and chemistry, experience and progress, and quality and quantity, must all be integrated together in order to render agriculture ecologically and economically viable” Baggiolini (translated from the original Italian).

This thesis is rooted in the perspective of the sustainable integration of scientific and biological facets in the process of vineyard production in southern Switzerland. In addition, it is hoped that this work may make a contribution towards the strengthening of the participative and integrative process that reflects the social interests in the region in question in this study.

1.4 Aim and outline of the thesis

The main focus of this thesis is twofold: first, to define which type of factors (management regime, environmental characteristics, biological interactions and landscape composition, and configuration) affect species composition and their related functional traits, and second, to root issues related to biodiversity and pest management within the framework of sustainable production.

Chapter 2: Management pressure drives leafhopper communities in vineyards in Southern Switzerland

In 2009, a pilot study was conducted that provided useful preliminary insights for the present study. Twenty-four study transects in total from the 8 main viticultural areas in Southern Switzerland were set up. Leafhoppers were used as taxa model to investigate the relative contribution of several abiotic factors that influence taxonomic and functional biodiversity. The study's outcomes facilitated the following for the present investigation:

- the selection of the most effective sampling techniques with respect to arthropods
- the development of the main study design
- the assessment of proposed statistical analysis techniques
- the development of further research questions and hypotheses.

Chapter 3: Determinants shaping community assemblages and species co-occurrence patterns between trophic levels

In 2011, we investigated the relative importance of environmental factors and biotic interaction shaping community assemblages by applying a multi-analytical approach. Analyses were conducted on two taxa: plants (producers) and leafhoppers (phytophagous), in vineyard agroecosystem in southern Switzerland. Results of this study were useful to generate hypotheses on the mechanisms underlying the coexistence between species observed both to local and regional scale.

Chapter 4: Indicators for taxonomic and functional aspects of biodiversity in the vineyard agroecosystem of Southern Switzerland

Selecting reliable indicators is a crucial step in assessing the effectiveness of agri-environmental schemes with respect to biodiversity conservation and its associated services. Ecological direct payments (subsidies) to promote a high level of biodiversity in Swiss vineyards are only granted for high quality vineyards whose value is calculated based on a list of plant species of particular interest. In order to identify indicator plant species associated with high levels of both taxonomic and functional biodiversity, a two-step multivariate analysis approach was applied.

Chapter 5: Comment évaluer la qualité botanique des surfaces agricoles de promotion de la biodiversité? L'agroécosystème viticole au Sud des Alpes suisses comme cas d'étude

How should the promotion of the botanical quality of farmland biodiversity be evaluated? The vineyard agroecosystem in Southern Switzerland case study.

In Switzerland, subsidies to provide ecological services are granted to 16 types of land uses for the promotion of biodiversity. The system whereby quality value is attributed varies according to the kind of surface. In any case, for vineyards, available instruments used in such evaluations appear to be partially inadequate and, at any rate, not immediately applicable to the diverse biogeographical contexts across Switzerland. For this reason, a conceptual framework to select reliable indicator species based on different criteria is proposed.

Chapter 6: A regional-scale survey to define the known and potential vectors of grapevine yellow phytoplasmas in vineyards South of Swiss Alps

Management practices in vineyards may affect biodiversity inside fields as well as in their surroundings. Nevertheless phytosanitary concerns can impose mandatory measures with the aim to eradicate the spread of pests. This is the case with phytoplasmas that cause grapevine yellow diseases. In this chapter, an evidence-based approach for the management of phytoplasma diseases in vineyards is outlined, in order to obtain information on the occurrence of phytoplasmas in insects.

Chapter 7: General discussion

The specific outcomes achieved throughout fundamental research into factors that affect biodiversity in vineyards have led to practical implications related to sustainable management in vineyards. The relationship between basic and applied scientific research is discussed.

1.5 Additional scientific papers and conferences

This thesis is based on 5 original articles, but also additional scientific papers (5 faunistic and 1 floristic) and 7 outreach articles. Overall 4 conference presentations given by myself arose from the present study (see Appendix 1:A1).

1.6 Description of study area

1.6.1 Location

The study area is located in Southern of Swiss Alps and the wine-growing area comprises an area of about 1’100 ha which produce about 50’000 hectoliters of wine annually (Office de statistique du canton du Tessin 2003-2014). Vineyards are generally small (ranges 3-6 hectare), on steep slopes and scattered throughout the territory, usually with a permanent natural green cover and more rarely with bare soil due to mechanical and/or chemical weed control. The insubric climate of the study region is influenced by the presence of lakes and alpine ranges which defined climatic conditions characterized by winters, which are normally dry and sunny, sometimes windy (Foehn from the North) and with periods of snow cover. The mean annual precipitation ranges from 1600 (S) to 1700 mm (N), and mean monthly temperatures from 0.5 (N) to 1.6 °C (S) in January and from 21.2 (N) to 23.5 °C (S) in July (Spinedi & Isotta 2004). Nowadays, more than 80% of vineyards planted in the region are dominated almost entirely by Merlot; it is a non-autochthonous cultivar which was introduced in southern Switzerland in the early 20th century, mainly because of the phylloxera outbreak. In the same period the specialized grape growing has slightly replaced mixed cultivation systems (grapevine, oats, barley and wheat) which have been gradually abandoned (Rossi 1908).

1.6.2 Selection of vineyards: study design

Forty-eight vineyards were chosen according to a stratified random selection process across the study area (Figure 1:1) by means of vector data of the land use (Vector25, Swisstopo) and a georeferenced geographical system (ArcGis 10). With the aim to capture the higher variability between vineyards, three main factors were considered in the selection of sites: slope, aspect and type of surrounding landscape unit. In Figure 1:1 a scheme of study design with a detailed description of selection process (Box 1).

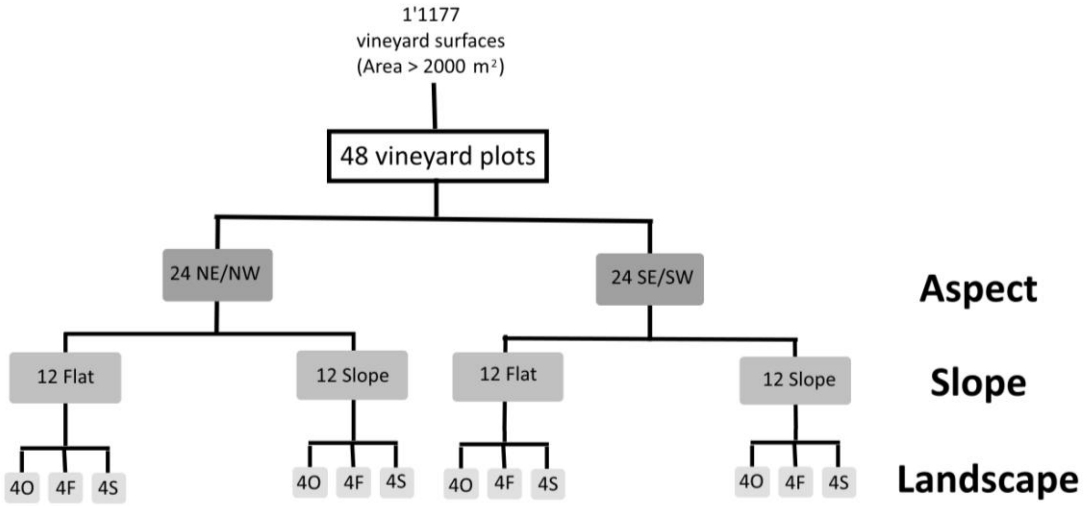
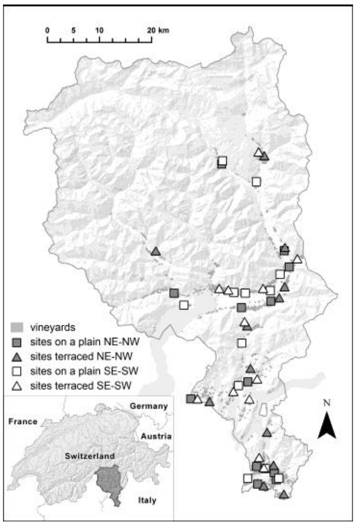


Figure 1:1 Study design for the vineyards selection in the Southern Swiss Alps region.



Box 1. Stratified random selection process of study vineyards.

A total of 1’177 vineyard surfaces with a minimum area of 2’000 m^2 were selected by means of VECTOR25 (Swisstopo, 2013). The 48 vineyards were randomly chosen within three nested categories. The first level of splitting was based on aspect of the surface with two groups of vineyards: twenty-four vineyards north-facing (NE/NW) and twenty-four south-facing (SE/SW). The second nested splitting was based on slope of the surface: Flat and Slope; the first one encompasses sites with slopes less than 5°, the second one encompasses sites with slopes more than 10° including terraced vineyards only. The third level of splitting regards the type of landscape unit dominating in the area of 500 m of radius around the study vineyards, each aspect-slope group was separated in three sub-groups: vegetated open area (O), forest (F) or settlements (S). Location of the studied vineyards is reported in the map on the left and in Chapter 4 (Appendix 4:A1).

1.6.3 Taxa target

I used plants and arthropods as target taxa because they are known to be reliable bioindicators for some important reasons: may reflect trends in species richness and community composition, cost-effective to use, are linked through feeding relationships, their small size with short generation time (arthropods) and sedentary (plants) makes them sensitive to environmental changes, etc. (for an overview see Gerlach, Samways and Pryke (2013). I chose eight taxa overall representing 4 trophic levels: vascular plants (as primary producer); Auchenorrhyncha (Hemiptera: Fulgoromorpha and Cicadomorpha) and Curculionoidea (Coleoptera) (as herbivores); Carabidae (Coleoptera) and Araneae (as predators), and Isopoda and Diplopoda (as detritivores).

Biological inventories of target taxa were obtained by applying a stratified sampling scheme which provided accuracy in the sampling and statistical validity. Vineyards were ideally divided in 3 zones tracing the configuration in parallel strips typical for the vineyard: flat inter-row spacing (**I** width ranging from 155 to 185 cm), on-row spacing (**R** part of vineyard floor below the vine canopy with a standard width of 50 cm) and sloped inter-row spacing (**S** always permanently covered with natural vegetation and sometimes with stone walls) (see the scheme in figure 1:2). The aforementioned zones were sampled with different sampling methods.

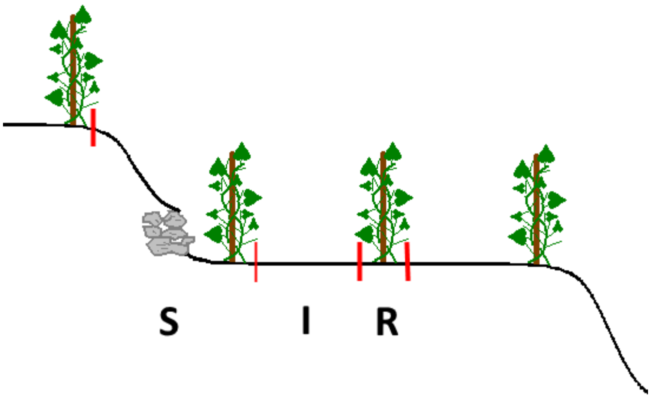


Figure 1:2 Representation of a "vineyard model" with 3 zones: **I** flat inter-row spacing, **R** on-row spacing and **S** sloped inter-row spacing.

Vascular plants

Two vegetation surveys were conducted in June and August in 2011. In this two periods most of early spring and late summer flowering plants are present. Species percentage cover of vascular plants was estimated within five 1m x 1m grids for each zone (relevès) randomly distributed over each vineyard within an area of 2000 m^2. Londo's relative cover value (percentage of cover) was assigned for each individual species according to the slightly modified Londo cover-abundance scale (Londo 1976). The grid was placed five times in each zone, and ten relevès (5 from inter-row and 5 from row) in flat vineyards and fifteen relevès (5 from inter-row , 5 from row and 5 from slope inter-row) in terraced vineyards were performed. Overall, 1'200 relevès were collected throughout the vegetation surveys. Species nomenclature follows Lauber & Wagner (2009).

Arthropods

Sampling of arthropods was carried out in 2011 over eight periods at monthly intervals, from April to October, covering the main activity period of arthropods (Hatley & Macmahon 1980; Brandmayr & Brandmayr 1986; Alikhan 1995; Stewart 2002). Based on a pilot survey (Trivellone *et al*. 2012) four effective sampling techniques were selected with the aim to intercept the total diversity in terms of number of species (Marshall & Canada 1994; Yi *et al*. 2012). Each sampling device was operated within an area 2000 m^2 at the center of the vineyard.

1. Pitfall trapping. Two pitfall trap stations were installed 20 m from field edges and 10m from each other, ensuring statistical independence of samples. A pitfall trap station consisted of four 200ml cups (diameter= 7 cm; height = 12 cm) placed along the vine row space and inserted into the ground 0.5 m apart. Each cup was half-filled with a saline (NaCl) solution and covered by a transparent PVC-roof. The traps were opened one week per month for a total of eight sampling periods. This kind of trap was exploited mainly to collect predators and detritivorous (e.g. Carabidae, Aranea, Isopoda, Diplopoda).

2. Yellow sticky trapping. Two yellow sticky traps were vertically placed in the vine canopy close to the pitfall trap station. Traps were opened during one week per month. Yellow sticky traps were used mainly to collect the arthropods strictly linked with grapevine. Yellow seems to be the best colour for trapping the arthropods, even if various kinds of insects react differently to different colours. However, bright yellow effectively collects most of winged "Homoptera" and parasitic Hymenoptera (Gibb & Oseto 2006).

3. D-vac suction sampling. The vegetation of the vineyard floor was sampled one time per month with a D-Vac suction sampler (D-Vac Suction Sampler Stihl SH 86 modified by EcoTech® https://www.ecotech-bonn.de/de/, with an opening diameter of the suction tube of 15 cm) and the device was operated for 120 seconds/sample and two samples were collected from the inter-row and the slope vegetation, respectively. The D-vac sampler was employed to sampling the arthropods associated with the vineyard floor vegetation. However, this technique is not suitable for large and heavy individuals which are underestimated (Mommertz *et al*. 1996).

4. Beating tray sampling. Thirty vine branches per sample were hand-shaked over an entomological umbrella (1m x 1m), collecting all arthropods fallen down. A total of two samples per vineyard were collected.This kind of umbrella has more surface area than the classical entomological net for sweeping and it is particularly suitable for cultivated arboreal plants and for all insects with low fly capability and all insects with an instinctive propensity to simulate death (White & Peterson 1998). Sixteen sites with a high percentage of surrounding forest were chosen to test the hypothesis that the presence of forests could influence the biodiversity of arthropods as well as the degree of insect pest infestation. In this aim, along a selected ecotone zone between vineyard and forest , the first three sampling techniques were employed and one sampling station was established.

Sixteen sites with higher percentage of forest in the surroundings were chosen and one sampling station was established along the buffer ecotone between vineyard and forest. Overall, 3’336 samples were collected throughout the study. All the collected individuals were grouped at the Order level, labeled and conserved in 70% alcohol and sent to specialists for identifications of the adult specimens to the species level. I identified all adults of Auchenorrhyncha group.

1.6.4 Abiotic factors

The selection of abiotic factors used as explanatory variables was based on the ecology of the studied taxa and on the knowledge acquired from the literature (Joern and Laws (2013) for a review). Overall, 36 variables grouped in 6 categories (management, topography, chemical and physical property of soil, plant structure of wild vegetation cover, composition of landscape in a 200 m radius and composition of landscape in a 500 m radius) were considered. An overview of the selected environmental variables is reported in Chapter 3 (Appendix 3:A2).

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Appendices

Material 1:A1. List of additional scientific papers (5 faunistic and 1 floristic), 7 outreach articles and 4 conference presentations arising from the present study.

Additional non-ISI papers

1. Cara C., Milani M., Trivellone V., Moretti M., Pezzati B., Jermini M. 2013. La minatrice americana della vite (Phyllocnistis vitegenella Clemens): dinamica delle popolazioni e potenziale di biocontrollo naturale in Ticino. Bollettino della Società ticinese di scienze naturali, 101: 75-80.
2. Trivellone V., Pedretti A., Caprani M., Pollini Paltrinieri L., Jermini M., Moretti M. 2013. Ragni e carabidi dei vigneti del Canton Ticino. Bollettino della Società ticinese di scienze naturali, 101: 63-72.
3. Hänggi A., Stäubli A., Heer X., Trivellone V., Pollini Paltrinieri L., Moretti M. 2014. Eleven new spider species (Arachnida: Araneae) for Switzerland discovered in vineyards in Ticino - What are possible reasons?. Bulletin Mitteilungsblatt SEG-SSE, 87:215-228
4. Germann G., Trivellone V., Pollini Paltrinieri L., Moretti M. 2013. First record of the adventive weevil Gymnetron rotundicolle Gyllenhal, 1838 from Switzerland (Coleoptera, Curculionidae). Bulletin Mitteilungsblatt SEG-SSE, 86: 1-5.
5. Trivellone V., Knop E., Turrini T., Andrey A., Humbert J.-Y., Kunz G. 2015. New and remarkable leafhoppers and planthoppers (Hemiptera: Auchenorrhyncha) from Switzerland. Bulletin Mitteilungsblatt SEG-SSE. 88: 273-284.
6. Bellosi B., Trivellone V., Jermini M., Moretti M., Schönenberger N. 2013. Composizione floristica dei vigneti in Ticino. Bollettino della Società ticinese di scienze naturali, 101: 55-60.

Outreach articles

1. Bogyo D., Vilisics F., Moretti M., Trivellone V. 2013. Isopoda and Diplopoda fauna of vineyards in Southeast-Switzerland. 12th Central European Workshop on soil zoology, April 8th-11th 2013, České Budějovice (Czech Republic). Abstract book: 14.
2. Trivellone V., Moretti M. 2013. Vigneti della Svizzera italiana: una fonte importante di biodiversità. Forestaviva, 52, 8.
3. Moretti M., Trivellone V. 2013. Découverte de nouvelles espèces d'invertébrés dans les vignobles du Tessin. Revue suisse de Viticulture, Arboriculture, Horticulture, 45(6): 377.
4. Trivellone V. 2014. I margini tra bosco e aree coltivate: un'opportunità per la valorizzazione della biodiversità in Ticino. Agricoltore Ticinese, 6, 13.

5. Trivellone V., Jermini M., Moretti M., Cara C. 2015. Interfaccia bosco-aree agricole: un mosaico paesaggistico ricco di parassitoidi utili. Agricoltore Ticinese, 36, 13.

6. Jermini M., Trivellone V. 2015. Combiner les modes d'échantillonnage pour affiner les strategies de lute. Revue suisse de Viticulture, Arboriculture, Horticulture, 47(4): 213.

7. Trivellone V., Moretti M. 2015. Weinberge im Tessin enthüllen unerwartet hohe Biodiversität. Diagonal, 1, 20-21.

Conference presentations

1. Trivellone V., Jermini M., Angelini E. 2015. Occurrence of Leaf- and Planthoppers known and potential vectors of phytoplasmas in vineyards of Southern Switzerland. IOBC-WPRS Conference of Working Group on "Integrated Protection and Production in Viticulture", Vienna, October 2015. Abstract Book and talk.

2. Trivellone V., Filippin L, Jermini M., Angelini E. 2015. Molecular characterization of phytoplasma strains in leafhoppers inhabiting the vineyards agroecosystem in Southern Switzerland. 3rd International Phytoplasmologist Working Group Meeting. Mauritius, January 2015. In: Phytophatogenic Mollicutes, 5(1): S45-S46. DOI: 10.5958/2249-4677.2015.00018.3 and talk.

3. Trivellone V., Pedretti A., Caprani M., Pollini Paltrinieri L., Jermini M., Moretti M. 2013. Arthropods as bio-indicators in vineyard agroecosystem. IOBC/WPRS Meeting of the Working Group "Integrated Protection and Production in Viticulture", Ascona (Switzerland), 13th - 17th October, 2013. Abstract Book: 121 and talk.

4. Trivellone V., Schönenberger N., Bellosi B., Jermini M., de Bello F., Mitchell E.A.D., Moretti M. 2013. How to select indicator plant species for taxonomic and functional biodiversity in the ecosystems affected by humans. XXIII Congresso Società Italiana di Ecologia, Ancona, 16th-18th September, 2013. Exstended abstract and talk.

Chapter 2 Management pressure drives leafhopper communities in vineyards in Southern Switzerland

Metcalfa pruinosa (Say, 1830) on grapevine shoot (photo: V. Trivellone)

Valeria Trivellone, Lucia Pollini Paltrinieri, Mauro Jermini and Marco Moretti

Published in *Insect Conservation and Diversity*, 5, 75–85, 2012

Abstract

1. The effects of the current changes in traditional agricultural practices in the Alps on the biodiversity affecting ecosystem functions and services are little known. Vineyards are among the oldest anthropogenic environments of high cultural and natural value that shape the landscape of large areas in Central and Southern Europe. In several mountain regions of the Alps, vineyards are a valid alternative to the landscape homogenization that has followed post-cultural land abandonment and agriculture intensification. Key unanswered questions remain regarding the relative contribution of several factors that influence biodiversity, and the level in management pressure with regards to taxonomic and functional diversity enhancement.

2. To answer these questions, we sampled leafhoppers (Auchenorrhyncha) as a model taxon using different standard techniques along 24 vine transects within 8 vineyard complexes in Southern Switzerland. Each transect included one vine row, vine canopy, its interrow, and the adjacent slope; the latter two were permanently grass-covered. Data were analyzed using a four-step approach.

3. Environment (5 variables) and Management (4 variables) accounted for most of the variance in the leafhopper assemblage. Pesticide use (insecticide and herbicide) and slope mowing are the most important management predictors of leafhopper species composition.

4. With increasing management pressure (i.e. pesticide and mowing), the number of indicator species and particularly the specialists (i.e. stenotopic and oligotopic species) decreases dramatically.

5. To promote taxonomic and functional complexity of communities in vineyard systems, we suggest low management pressure with moderate use of pesticide and a low intensity regime of slope mowing.

Keywords Auchenorrhyncha, insecticide, biodiversity, conservation, indicator species, functional traits, grassland, invertebrates.

Introduction

Global agricultural policy is undergoing significant changes towards new approaches that take into account the multifunctional concept (IAASTD, 2008). In this perspective, the conservation of both natural resources and ecosystem services is fundamental to provide the indispensable base for the production of essential goods and services for human survival (Díaz *et al.,* 2007). Biodiversity is a necessary underlying component of goods and ecological services and land-use practices, especially in grassland ecosystems, have been identified as the single major cause of biodiversity loss in recent years (Chapin *et al.,* 2000; Vile *et al.,* 2005; Díaz *et al.,* 2006; Kremen *et al.,* 2007). In particular, grasslands in the Alps are currently going through a series of profound changes with unknown consequences on both biodiversity and related ecosystem functions and services. In the last few decades, human activity has modified the landscape and biodiversity in the Alps through intensification of agricultural practices in some areas as well as abandonment of traditional practices in others (e.g. Chemini & Rizzoli, 2003; Sergio & Pedrini, 2007; Fischer *et al.,* 2008).

The vineyard is a valuable element of alpine landscape shaped by cultural traditions and natural conditions. By adopting ecological management, it is possible to preserve biodiversity and increase the stability and resilience of the agroecosystem while also maintaining the benefit drawn by farmers. Several studies have shown that farming practices and management regimes of vineyard grasslands are the most important factors determining biodiversity of plants and invertebrates (e.g. Di Giulio, *et al.*, 2001; Costello & Daane, 2003; Ponti *et al.*, 2005; Thomson & Hoffmann, 2007; Sharley *et al.*, 2008; Bruggisser *et al.*, 2010). Other factors that might contribute to biodiversity enhancement and structuring in vineyard systems and in vineyard grasslands in particular are local environmental conditions (especially in mountain regions) and the spatial arrangement of the locations (ecological connectivity). Schweiger *et al.* (2005) suggested that management effort should be focused on habitat connectivity and land-use intensity, which are the factors that account for most of the variability of arthropod communities in several agricultural landscapes.

Central to understanding community distribution and biodiversity in grassland systems in mountain regions is knowledge of the relative importance and interaction between management practices, local environmental conditions and the spatial arrangement of the locations. In particular, our study aimed (i) to assess the relative contribution of management, environment and space variables on the invertebrate community assemblages of the vineyard system; (ii) to examine the effect of different management measures on invertebrate species composition; (iii) to define indicator species of grass-covered vineyard under different management practices and to characterize them from a functional perspective; (iv) to propose management guidelines to enhance taxonomic and functional diversity in vineyard grasslands in the Alps.

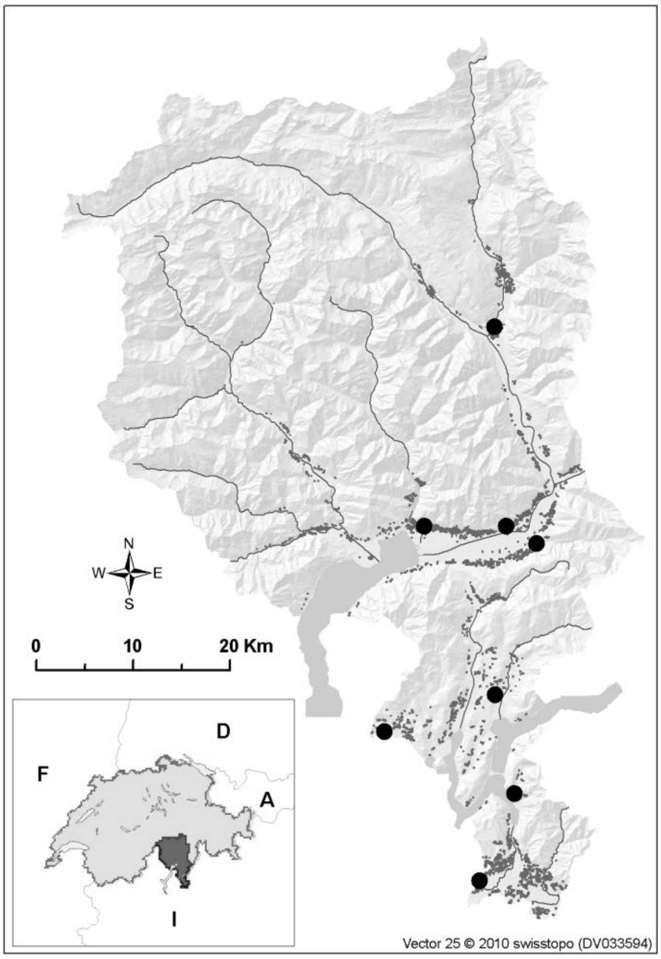
To answer to these points, we selected Auchenorrhyncha (Hemiptera: Fulgoromorpha and Cicadomorpha), leafhoppers hereafter, as our model taxon, as it represents an important taxonomic group of both conservation and agronomic concern in vineyard systems. Leafhoppers are widely used as indicators of changes in management and composition of grassland systems (see Biedermann *et al.*, 2005 for a review).

Materials and methods

Study area and sampling design

The study was carried out in the main vineyard region of Southern Switzerland, along a North-South gradient from Biasca (46°21'N–8°57'E) to Stabio (45°51'N–8°55'E), Canton Ticino (Fig. 2:1; Pythoud, 2007 for details). The study area has a moist, warm temperate climate, with a mean annual precipitation ranging from 1600 (S) to 1700 mm (N), and mean monthly temperatures from

0.5 (N) to 1.6 °C (S) in January and from 21.2 (N) to 23.5 °C (S) in July. Vineyards are mainly located along south-facing steep terraced slopes (256-436 m a.s.l.) with grapevine rows along slope lines. Vineyards are often composed of small areas scattered at different suitable sites but grouped in geographical units (vineyard complexes), which are divided by morphological or anthropogenic structures and surrounded by settlements, gardens, semi-natural open habitats and forest edges.



Vector 25 © 2010 swisstopo (DV033594)

Figure 2:1 Location of the eight vineyard complexes (black dot) selected for our study within the vineyard region (dark-grey areas) of Southern Switzerland.

Data sampling

We designed the data sampling to include between- and within- vineyard variability, as our case study. In the study area, we selected 8 vineyard complexes, 4 in the southern and 4 in the northern part of the main vineyard region, to maximise the geographical variance between vineyard complexes (distance between vineyards: minimum 9 km; maximum 21 km) as an important source of variation of biotic and abiotic conditions. Within each vineyard complex, we selected three 20 m x 6 m sampling transects (*transect* hereafter) consisting of one vine row, vine canopy, interrow and adjacent slope (if present). The latter two were permanently covered by herb layer, thus grassland vegetation cover constituted the main environment within our vineyard system. The three transects were located in the upper, middle and low sector of each vineyard complex (distance between transects: minimum 20 m; maximum 40 m) to include the within-vineyard complex variability given by their particular geomorphological conditions. There were 24 transects in total.

In each transect, leafhoppers were sampled from 4 May to 29 July 2009 for a total of four sampling periods, covering the main activity period of leafhoppers in vineyards. We used three standard methods that permitted the sampling of species from different life forms and strategies (see Stewart, 2002 for a review). Species with low mobility (i.e. brachypterous and ground-dwellers) were sampled using pitfall traps, which consisted of 3 plastic beakers (opening diameter 75 mm) recessed into the soil and arranged in a line, at a distance of 50 cm, in the middle of the transect and filled with a saturated salt solution and some drops of detergent as a surfactant. Vacuum aspiration (D-Vac Suction Sampler Stihl SH 86 modified by EcoTech®; http://www.ecotech-bonn.de/, with an opening diameter of the suction tube of 15 cm; 120 seconds on 60 sampling points per transect) and sweep netting (opening diameter of 35 cm; 80 sweeps per transect) were used to sample species living on the low and upper grass layer, as well as on the vine canopy along the transects. Pitfall trap, vacuum and sweep net samples were collected once every 3-4 weeks during the sampling period.

Additionally, we sampled three groups of explanatory variables in each transect (Tab. 2:1), including five environment variables (i.e. aspect, slope of the transect, altitude, presence of vineyard slopes and vegetation type), four management variables (i.e. mowing of the slope, mowing of the interrow, application of insecticide and application of herbicide) and three spatial variables (see next section).

Table 2:1 List of environmental, management and spatial variables forming the initial pool of predictors used to model the community composition of leafhoppers.

Group of variable	Code	Type of variable	Description
Environment			
Aspect	ASPECT	Continuous*	$X_{\mathrm{tr}\,=}\cos[\mathrm{radiant}\,(X - 45^\circ)] + 1$ (Beers *et al.*, 1966)
Slope of the transect	SLOPE	Continuous*	
Altitude	ALT	Continuous*	
Presence of vineyard slopes	VINEYSLOPE	Binary	0 = absence; 1 = presence
Vegetation type	RUDVEG	Binary	0 = dry meadow; 1 = ruderal
Management			
Mowing of the slope	MOWSLOPE	Binary	0 = no; 1 = yes
Mowing of the interrow	MOWINTER	Binary	1 = 2-3 cuts per year; 2 = 4-5 cuts per year
Application of insecticide	INSECTIC	Binary	0 = no application; 1 = 2 applic. per year on the vine canopy
Application of herbicide	HERBIC	Binary	0 = no application; 1 = 2 applic. per year on the vine row
Space			
Moran's eigenvectors map	MEM	Continuous	Three selected eigenvectors after Dray *et al.* (2006) (see section Spatial data)

* Data calculated on the basis of the 25 x 25 Digital Elevation Model (DEM25, Federal Office of Topography – Swisstopo)

Spatial data

To consider the influence of the spatial arrangement on leafhopper assemblages at both small and large scales, we used the Moran's eigenvector maps (MEMs) approach. This technique belongs to the Principal Coordinates of Neighbour Matrices family of analyses, and was first proposed by Borcard and Legendre (2002) and further developed by Dray *et al.* (2006). It is increasingly used to assess the spatial influence on community structure in ecological studies. MEMs are constructed from a spatial weighting matrix (**W**) calculated by the Hadamard product of a connectivity matrix (**B**) by a weighting matrix (**A**). The **B** matrix is based on spatial coordinates while the neighbourhood between transects is constructed using the distance criteria of *nearest neighbors*. Finally, Moran's eigenvectors and eigenvalues are calculated on the spatial weighting matrix, and the eigenvector matrix that explains the largest part of the leafhopper community is selected. For more details, see Dray *et al.* (2006) for the mathematical aspects, and Sattler *et al.* (2010) for an application.

Species and species traits

All adult leafhopper specimens were identified at species level by the first author. Nomenclature follows Ribaut (1936, 1952), Della Giustina (1989), Holzinger *et al.* (2003) and Biedermann and Niedringhaus (2009). Voucher specimens of each species are deposited in the Natural History Museum of Lugano, Switzerland.

Each species was described in terms of four traits (i.e. Diet width, Overwintering stage, Voltinism and Dispersal capacity; see Appendix – Table 2:A1) after Nickel and Remane (2002) and Nickel (2003). According to the classification of grassland Auchenorrhyncha proposed by Achtziger and Nickel (1997) and Nickel and Achtziger (2005), different combinations of ecological traits defined four groups (Pioneer, Eurytopic, Oligotopic and Stenotopic) of synthetic life strategies with differential responses to management.

The Pioneer and Eurytopic species are defined as generalists and Oligotopic and Stenotopic species as specialists.

Data analyses

We used four complementary statistical methods to answer our questions (see a-d in Fig. 2:2 for an overview).

To quantify the relative contribution of the three sets of variables (Management, Environment and Space; see Table 2:1) we hierarchically partitioned the variability in the community data of the 24 transects (see *a* in Fig. 2:2) (Borcard *et al*., 1992; Anderson & Gribble, 1998; Legendre & Legendre, 1998). All the management and environmental variables were included in the analysis after the forward selection by Dray *et al*. (2007) (P = 0.05 after 9999 random permutations) and the double-stopping procedure by Blanchet *et al.* (2008) did not eliminate any variables. The variation explained in each Redundancy Analysis (RDA) model was reported as the adjusted coefficient of multiple determination R^2 (R^2_{adj}), which takes the number of predictor variables and sample size into account to prevent the inflation of R^2 values (Peres-Neto *et al*., 2006). Singletons had been removed from the data matrix before analyses to eliminate the effects of vagrant species that are not closely related to the agrosystem vineyard, while for the analyses (if not otherwise indicated) we used the Hellinger transformation to reduce the influence of extreme values and the effect of the double-absences in the data matrix (Legendre & Gallagher, 2001).

The relationship between the leafhopper assemblage and explanatory variables (Management and Environment) was investigated by partial redundancy analysis (pRDA) on data files (see *b* in Fig. 2:2) using Space (MEMs) as co-variables to remove the confounding effect of space. The significance of the different canonical axes was assessed by Monte Carlo permutation tests ($P < 0.05$ after 9999 random permutations).

Multivariate Regression Tree (MRT) analysis was used to relate abundances of leafhopper species to management variables and create groups of transects (see *c* in Fig. 2:2). Each split minimises the dissimilarity (sum of squared Euclidian distances, SSD) of the species and transects within the clusters. Each of them is defined by an explanatory variable value (De’aht, 2002). For the analysis, we used spatially detrended leafhopper data to remove the spatial component form the grouping.

We finally used indicator species analysis (Dufrêne & Legendre, 1997) to investigate management preferences of species taken individually (see *d* in Fig. 2:2), by testing their specificity and fidelity to transect groups (*sensu* Dufrêne & Legendre, 1997; De Cáceres *et al*., 2010) resulting from the MRT. Indicator species were selected based on their indicator value (IndVal) and *P*-value (<0.05) after 9999 random permutations and Holm correction for multiple tests (De Cáceres *et al*., 2010). The data species were log ($x + 1$) transformed.

All statistical analyses were performed using R 2.10.1 (R Development Core Team, 2009).

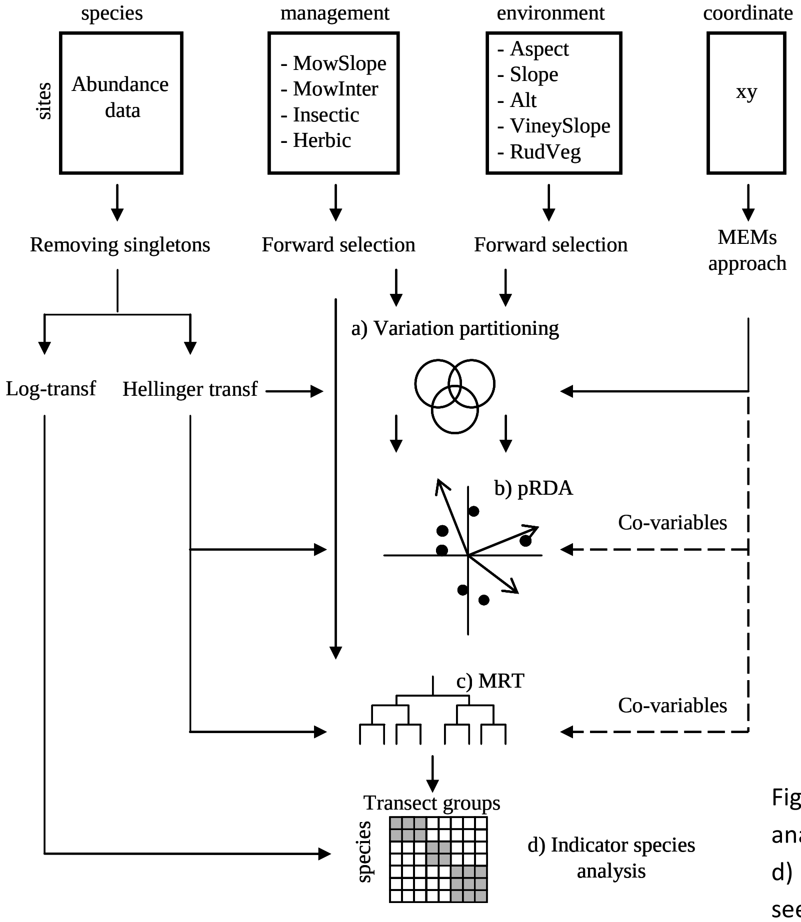


Figure 2:2 Diagram showing the statistical analyses based on a four step approach (a–d) (see Materials and methods), for details see Table 2:1.

Results

Altogether, we sampled 12 946 individuals (9529 adults and 3417 unidentified juvenile forms) belonging to 106 species. The leafhoppers *Arocephalus longiceps* (12.2%), *Jassargus bisubulatus* (9.5%), *Cicadella viridis* (7.8%), *Anaceratagallia ribauti* (6.8%), *Dicranotropis hamata* (5.5%), *Reptalus cuspidatus* (5.1%) were most abundant and the first species was observed twice as often as the last. Approximately 78% of the community is associated with the herb layer, while none of the dominant species is strictly associated with vine canopy (ampelophagous species). Overall, 36 species (35%) were recorded as singletons (i.e. species that occurred in one sample only) and were accordingly removed from the analyses (see Materials and methods).

Factors affecting leafhopper composition

Variation partition of Management (four variables), Environment (five variables) and Space (three variables) accounted for 73% of the total variance (Fig. 2:3), while the pure effect of each set of variables was 21% for Environment, 13% for Management and 9% for Space. The greatest shared variation occurred between Management and Environment (19%); the overall shared fraction with Space was 13% (specifically 10% with Environment and 3% with Management).

Since Management and Environment accounted for 53% of the overall variance in the leafhopper community assemblage (Fig. 2:3), we selected these two sets of variables as predictors in the pRDA, while Space (i.e. Moran's eigenvector values, MEMs) was used as covariable (see Materials and methods).

Community response to Environment and Management

The pRDA (Fig. 2:4) showed that the first two axes accounted for 62.9% of the total variation in the leafhopper community assemblage; 82.6% was explained by the first four axes. The first canonical axis (37.3% of the variance) was negatively associated with both the slope of transects (SLOPE, -0.718) and the use of insecticide (INSECTIC, -0.669), while it was positively related to the occurrence of ruderal vegetation (RUDVEG, 0.474). The second axis (25.6% of the variance) was negatively associated with the use of herbicide (HERBIC, -0.820) and altitude (ALT, -0.783) and positively related to the presence of vineyard slopes (VINEYSLOPE, 0.658) and the slope of the transect (SLOPE, 0.507) (details are given in Appendix – Table 2:A2).

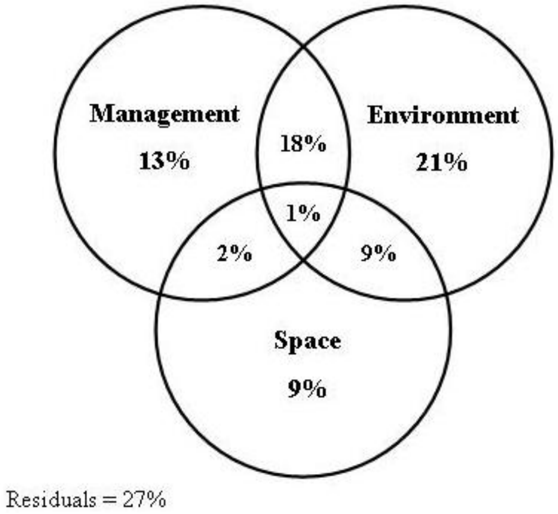


Figure 2:3 Variation partitioning (%) of the influence of three sets of explanatory variables (Management, Environment and Space) on leafhopper communities. All effects were significant. The variables of each set are listed in Table 2:1.

Very few species were associated with both insecticide (INSECTIC) [e.g. *Arocephalus longiceps* (*Ar.lo*) and *Zyginidia pullula* (*Zy.pu*)] and slope of transects (SLOPE) [*Aconurella prolixa* (*Ac.pr*) and *Muellerianella fairmairei* (*Mu.fa*)] (see left part of the biplot in Fig. 2:4). Most of the species [e.g. *Scaphoideus titanus* (*Sc.ti*); *Muellerianella extrusa* (*Mu.ex*); *Ribautodelphax albostriata* (*Ri.al*); *D. hamata* (*Di.ha*)] were, instead, associated with ruderal vegetation (RUDVEG) and the absence of insecticide (see right part of the biplot in Fig. 2:4). Several species [e.g. *Z. pullula* (*Zy.pu*); *A. ribauti* (*An.ri*); *Zygina rhamni* (*Zy.rh*); *Ebarrius cognatus* (*Eb.co*)] were positively associated with the use of herbicide (HERBIC) and aspect (ASPECT) along the second axis at the lower-left side of the biplot, while a small number of species [e.g. *C. viridis* (*Ci.vi*); *J. bisubulatus* (*Ja.bi*)] were negatively correlated with herbicide and aspect.

Effect of Management on taxonomic and functional aspects

Multiple Regression Tree analysis selected a five-leaf tree with four splits (Fig. 2:5) and a minimum estimated predictive error of 0.945 (relative error 0.608; variance accounted for 39.2%; proportion of the total sum of squares accounted for 29%). The first split was based on insecticide (INSECTIC) followed by two main branches and further splitting involving both the application of herbicide (HERBIC) and mowing of the slopes (MOWSLOPE); the latter as an overlapping variable in two distinct part of the trees.

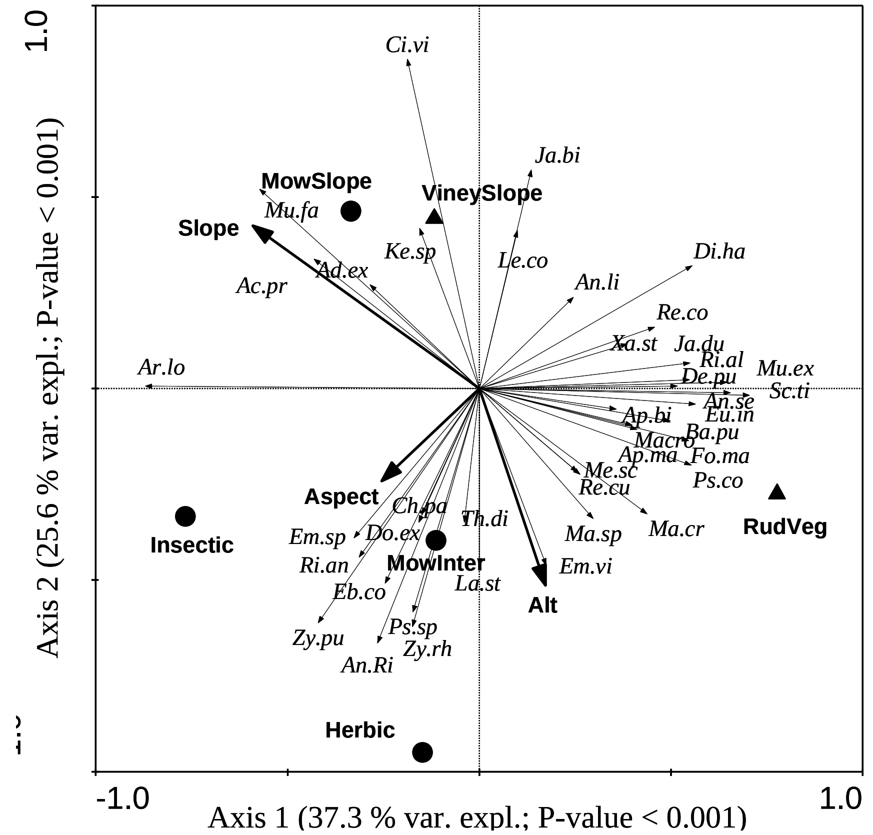


Figure 2:4 Partial Redundancy Analysis (pRDA) of leafhopper community response to environmental (▲ binary or → continuous) and management (•) variables using spatial variables (MEM's eigenvectors) as co-variables.

Only the species most correlated to the first two canonical axes (n = 41 out of 65) are shown. var.expl.: variance explained. See Table 2:1 for variable names. Species abbreviations contain the first two letters of the genera and first two letters of the species. *Ci.vi*: *Cicadella viridis*; *Ja.bi*: *Jassargus bisubulatus*; *Le.co*: *Lepyronia coleoptrata*; *An.li*: *Anoscopus* cfr. *limicola*; *Di.ha*: *Dicranotropis hamata*; *Re.co*: *Recilia coronifera*; *Xa.st*: *Xantodelphax straminea*; *Ja.du*: *Javesella dubia*; *Ri.al*: *Ribautodelphax albostriata*; *De.pu*: *Deltocephalus pulicaris*; *Mu.ex*: *Muellerianella extrusa*; *An.se*: *Anoscopus serratulae*; *Sc.ti*: *Scaphoideus titanus*; *Eu.in*: *Euscelis incisus*; *Ap.bi*: *Aphrodes bicincta*; *Ba.pu*: *Balclutha punctata*; *Macro*: *Macropsis* sp.; *Fo.ma*: *Forcipata major*; *Ap.ma*: *Aphrodes makarovi*; *Ps.co*: *Psammotettix confinis*; *Me.sc*: *Megophthalmus scanicus*; *Re.cu*: *Reptalus cuspidatus*; *Ma.cr*: *Macrosteles cristatus*; *Ma*.sp: *Macrosteles* sp.; *Em.vi*: *Empoasca vitis*; *Th.di*: *Thamnotettix dilutior*; *La.st*: *Laodelphax striatella*; *Ch.pa*: *Chlorita paolii*; *Do.ex*: *Doratura exilis*; *Em*.sp: *Empoasca* spp.; *Ri.an*: *Ribautodelphax angulosa*; *Eb.co*: *Ebarrius cognatus*; *Ps*.sp: *Psammotettix* spp.; *Zy.pu*: *Zyginidia pullula*; *Zy.rh*: *Zygina rhamni*; *An.ri*: *Anaceratagallia ribauti*; *Ar.lo*: *Arocephalus longiceps*; *Ac.pr*: *Aconurella prolixa*; *Ad.ex*: *Adarrus exornatus*; *Ke*.sp: *Kelisia* sp.; *Mu.fa*: *Muellerianella fairmairei*.

The Indicator species analysis used to select characteristic species associated with Management resulted in 27 (41.5%) species being significantly associated with one or more of the five MRTs' groups; 12 group combinations in total (see Table 2:2). Seven species were indicators of high management pressure (Gr.1-3), while 13 species were positively associated with the absence of insecticide and low management pressure (Gr.4-5). Seven species were characteristic of transect groups with both management types.

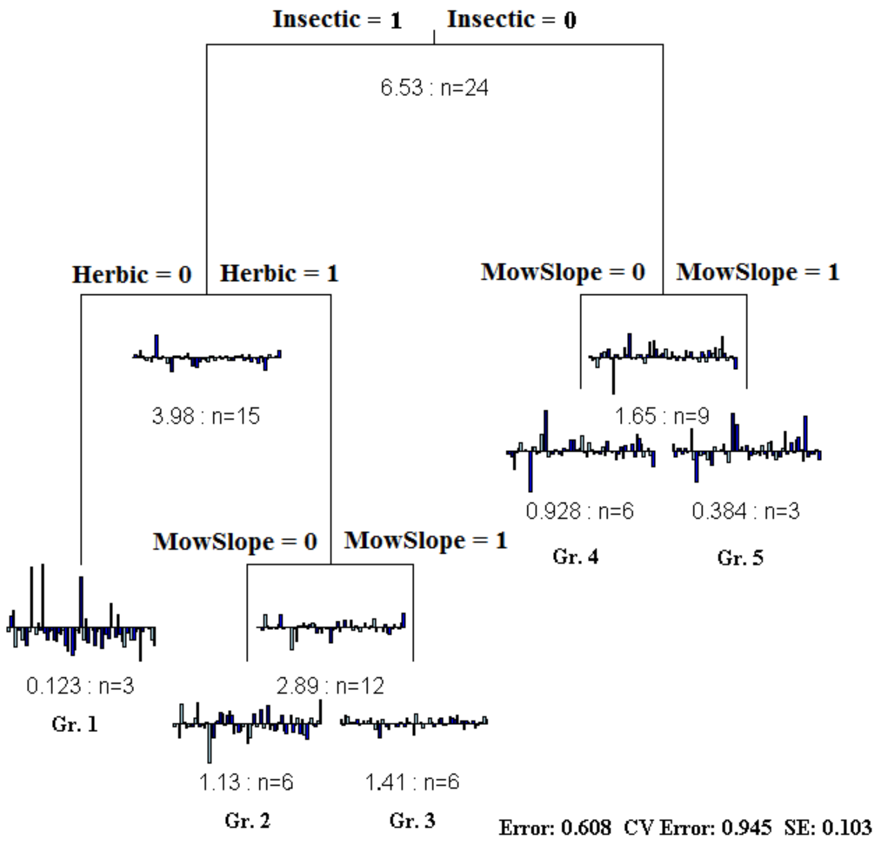


Figure 2:5 Multivariate Regression Tree (MRT) based on the leafhopper data constrained by management variables (for abbreviations, see Table 2:1). The five-leaf tree is pruned to five transect groups (Gr. 1–5). In each node or leaf the multivariate mean of transects (range from 0.123 to 6.53) and the number of transects grouped (*n*) are reported. CV Error, cross-validated

Table 2:2 shows that insecticide and increasing management pressure affect functional leafhopper community assemblage by removing specialists (i.e. stenotopic and oligotopic species) from the indicator species. Stenotopic species only occur in transect groups with the absence of insecticide and low management pressure, and altogether 77% of indicator species belonging to the specialist category. On the contrary, in the intensively managed transect groups, only 33% of indicator species are classified as oligotopic. The generalist species characterized (86%) the sites with both management types, with the dominance of pioneer species (50%). In Appendix – Table 2:A3, the details of ecological traits for each indicator species are given.

Table 2:2 Indicator species significantly associated with one or more groups of transects (Gr. 1–5) derived from the MRT analysis (see Fig. 2:5) based on the management variables insecticide (Ins), herbicide (Her) and mowing of the slopes (Mow).

Indicator species groups	**Species**	**LS**	**IndVal**	**p-val.**	**Gr 1** **Ins** **-** **-**	**Gr 2** **Ins** **Her** **-**	**Gr 3** **Ins** **Her** **Mow**	**Gr 4** **-** **-** **-**	**Gr 5** **-** **-** **Mow**
Species tolerating increasing management pressure	*Aconurella prolixa*	Eur	0.748	0.018	■				
	Kelisia sp.	-	0.691	0.037	■				
	Zyginidia pullula	Eur	0.787	0.001	■	■			
	Arocephalus longiceps	Eur	0.640	0.036	■	■	■		
	Psammotettix alienus	Pio	0.635	0.036			■		
	Cercopis vulnerata	**Oli**	0.621	0.039			■		
	Zygina rhamni	**Oli**	0.605	0.040			■		
Species related to low management pressure	*Dicranotropis hamata*	Eur	0.809	0.002				■	
	Hyalesthes obsoletus	**Oli**	0.645	0.040				■	
	Ribautodelphax albostriata	**Ste**	0.893	0.001				■	■
	Scaphoideus titanus	**Oli**	0.861	0.001				■	■
	Euscelis incisus	Eur	0.733	0.004				■	■
	Recilia coronifer	**Oli**	0.731	0.005				■	■
	Anoscopus cfr. limicola	**Oli**	0.614	0.032				■	■
	Eupteryx notata	**Oli**	0.920	0.002					■
	Psammotettix cephalotes	**Oli**	0.848	0.002					■
	Aphrodes makarovi	**Oli**	0.727	0.002					■
	Empoasca decipiens	Eur	0.698	0.018					■
	Graphocraerus ventralis	**Oli**	0.691	0.034					■
	Acanthodelphax spinosa	**Ste**	0.638	0.042					■
Species without clear management preferences	*Javesella dubia*	Eur	0.759	0.005			■	■	
	Macrosteles laevis	Pio	0.696	0.009			■	■	
	Macrosteles sp.	-	0.805	0.002		■	■	■	
	Psammotettix confinis	Pio	0.731	0.003		■	■	■	
	Laodelphax striatella	Pio	0.724	0.003		■	■	■	
	Emelyanoviana mollicula	Eur	0.596	0.031		■	■	■	■
	Cicadella viridis	**Oli**	0.734	0.004	■			■	■

Groups 1–3 include transects associated with the use of insecticide and an increasing management pressure; Groups 4 and 5 comprise transects without insecticide and herbicide and low management pressure. Life strategy (LS) describes the degree of species specialisation (Ste: stenotopic; Oli: oligotopic; Eur: eurytopic; Pio: pioneer) based on four functional attributes (see Achtziger & Nickel, 1997) as shown in Appendix - Table 2:A1. Specialist species (i.e. *Ste* and *Oli*) are written in bold. IndVal: indicator value; *P*-value <0.005 after 9999 permutations (see Materials and methods). The full list of the species can be requested from the first author.

Discussion

Factors influencing leafhopper community assemblage in vineyards

Our study showed that both Environment and Management account for most of the variance in leafhopper community composition in the vineyard region of Southern Switzerland. The portion of variance shared between the two sets of variables indicates that management effect is structured by the local environmental conditions such as slope, altitude and aspect. These probably have an influence on the micro-climate and local working conditions (especially on steep slopes), which are the main factors that determine the type, the intensity and the regime of management used. Our results are consistent with several authors who have shown that the effect of management practices on biodiversity and community composition is mediated by several other factors, such as aspect, light conditions, isolation (e.g. Di Giulio *et al*., 2001) or habitat type (e.g. Jeanneret *et al*., 2003).

On the other hand, the spatial arrangement of the transects has minimal effect on the leafhopper assemblage without structuring the effect of both Management and Environment. Our results are consistent with Schweiger *et al*. (2005) who suggest that in the absence of confounding effects of both small and large spatial geographical scales, conservation actions should be mainly targeted through decreased management pressure. In our study, management pressure was mainly due to the use of insecticide which affected leafhopper assemblage by both reducing the number of species and changing their relative composition. Similar results were obtained by Teodorescu and Cogălniceanu (2005) for spiders and carabid beetles in pesticide-treated crops in wheat fields in the southern plain of Romania and by Bruggisser *et al*. (2010) for grasshoppers in vineyard grasslands in SW-Switzerland.

Taxonomic and functional response to management intensity

Increasing management pressure, in particular by using insecticide and herbicide, negatively affects the composition both of leafhopper species communities and their life strategies. The number of indicator species in heavily treated grass-covered vineyard decreased and only a few species were highly tolerant to pesticide and frequent mowing. These species (such as *Psammotettix alienus* and *A. longiceps*) are highly mobile and are thus able to quickly colonize the managed area by taking advantage of the temporary lack of competition by the late successional stage species. By contrast, and consistently with Nickel and Achtziger (2005), our study showed that leafhopper specialists, i.e. stenotopic and oligotopic species (e.g. *Acanthodelphax spinosa* and *R. albostriata*) were very sensitive to treatment and cutting (Morris & Plant, 1983; Nickel & Hildebrandt, 2003) and are thus positively influenced by low management pressure in the vineyard grassland. Extensively managed transects include patches of structurally complex vegetation that allow many species with different ecological requirements to coexist. Generalists (i.e. pioneer and eurytopic species), such as *Laodelphax striatella* and *Psammotettix confinis* did not show any clear effects with regard to management, as also found by Achtziger *et al*. (1999) for distinct taxa in wet grassland systems in Southern Germany.

Conservation and practical implications

Vineyards have the primary function of producing wine. There is, however, a general consensus that vineyards play an important role in maintaining a diverse landscape mosaic and enhancing biodiversity in contrast to post-cultural landscape homogenization. Nevertheless, farmers must cope with two major concerns about vectors of phytoplasma associated with Flavescence dorée and Bois noir diseases. *Scaphoideus titanus* transmit to vine ‘*Candidatus* Phytoplasma vitis’ and *Hyalesthes obsoletus* transmit to vine ‘*Candidatus* Phytoplasma solani’ (Weintraub & Beanland, 2006). Until now management practices in Southern Switzerland, including an annual pest control program with at least two insecticide treatments, have mainly aimed to reduce these problematic species. Nevertheless, in some cases the treatment programs are not effective. Leafhoppers may show different behavioural patterns and different host plant range width depending on environmental conditions (including landscape composition) and management regime of the vineyard grassland (Novotný, 1994). To date, this remains an open issue and in our opinion a new approach based on an active-adaptive management should be considered (Shea *et al*., 2002; Baumgärtner *et al*., 2010). In doing this, suitable management practices should be proposed on different spatial scales on a case by case basis. These practices should consider ecological elements, such as refuges or alternative habitats for the key species, both pest and beneficial.

Furthermore, as highlighted by our study, the negative effect of intensive management of vineyard grasslands on the leafhopper community and functional composition is quite dramatic. Long-term impacts might negatively affect the taxonomic and functional diversity of communities, with possible negative affects on the natural defence dynamic provided by specific parasitoids and other beneficial organisms (e.g. Thomson & Hoffman, 2007; Sharley *et al*., 2008). Extensive management practices, especially along the slopes, are in fact likely to play a crucial role in preserving a high proportion of natural and semi-natural areas that provide refuge for different groups of invertebrates and their competitors (Duelli, 1997; Jeanneret *et al*., 2003), as well as scarce and rare species of conservational concern (Nilsson *et al*., 2008).

Overall, the results of our study suggest that management of vine canopies and vineyard grasslands should allow for the combination of both specific conservation programmes (i.e. protection of rare or endangered species) and socio-economic needs (i.e. control of pest species for sustainable wine production).

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Appendices

Table 2:A1. Description of leafhopper life strategy traits based on Nickel and Remane (2002) and Nickel (2003).

Life strategy traits	Trait code	Categories
Diet width	Monoph1	Monophagous on 1 plant species
	Monoph2	Monophagous on 1 plant genus
	Oligoph1	Oligophagous on 1 plant family
	Oligoph2	Oligophagous on 2 plant families or less than 5 species belonging to maximum 5 families
	Polyph	Polyphagous (for all other cases)
Overwintering stage	Egg	Egg stage
	Nymph	Nymphal stage
	Adult	Adult stage
Voltinism	Voltin	Number of generations/ year: 0.5 , 1, 2, > 2
Dispersal capacity	Brachy	Brachypterous
	Poly	Polymorph
	Macro	Macropterous
	Mesoph	Mesophyll

*The wing length classification of some species of Deltocephalinae is a simplification.

Table 2:A2. Environment and Management variables and correlation values with the first four canonical axes of the pRDA (Fig. 2:4). Correlation values higher than 0.475 are written in bold.

Explanatory variables	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
Environment				
RUDVEG	**0.476**	-0.165	0.263	-0.268
SLOPE	**-0.718**	**0.507**	0.143	0.046
VINEYSLOPE	-0.178	**0.658**	0.532	-0.127
ALT	0.269	**-0.783**	0.218	-0.212
ASPECT	-0.410	-0.381	-0.298	-0.090
Management				
INSECTIC	**-0.669**	-0.286	0.074	**0.613**
HERBIC	-0.130	**-0.820**	0.414	-0.027
MOWSLOPE	-0.231	0.313	0.435	-0.168
MOWINTER	-0.062	-0.211	-0.019	-0.011
Cumul. var. expl.	0.373	0.629	0.738	0.826
Eigenvalue	0.211	0.145	0.062	0.050
P-value	0.0001	0.0001	0.0001	0.0001

Table 2:A3. List of the indicator species (see Table 2:2) and their attributes with respect to four functional traits, diet width (DW); overwintering stage (OW); voltinism – number of annual generations (VL); dispersal capacity (DC) based on Nickel and Remane (2002) and Nickel (2003) (see Table 2:A1), synthesised in a unique trait, life strategy (LS) (see Achtziger & Nickel, 1997). Specialist species (i.e. *Ste* and *Oli*) are written in bold.

Indicator species groups	**Species**	**DW**	**OW**	**VL**	**DC**	**LS**
Species tolerating increasing management pressure	*Aconurella prolixa*	polyphagous	egg	1	macropterous	Eurytopic
	Kelisia sp.	-	-	-	-	-
	Zyginidia pullula	oligophagous1	adult	2	macropterous	Eurytopic
	Arocephalus longiceps	oligophagous1	egg	2	macropterous	Eurytopic
	Psammotettix alienus	oligophagous1	egg	2	macropterous	Pioneer
	Cercopis vulnerata	polyphagous	nymph	1	macropterous	**Oligotopic**
	Zygina rhamni	polyphagous	adult	2	macropterous	**Oligotopic**
Species related to low management pressure	*Dicranotropis hamata*	oligophagous1	nymph	2	dimorphic	Eurytopic
	Hyalesthes obsoletus	polyphagous	nymph	1	macropterous	**Oligotopic**
	Ribautodelphax albostriata	monophagous1	nymph	2	dimorphic	**Stenotopic**
	Scaphoideus titanus	monophagous2	egg	1	macropterous	**Oligotopic**
	Euscelis incisus	oligophagous2	nymph	2	macropterous	Eurytopic
	Recilia coronifer	oligophagous1	egg	1	macropterous	Eurytopic
	Anoscopus limicola	oligophagous1	egg	1	macropterous	**Oligotopic**
	Eupteryx notata	oligophagous2	egg	2	macropterous	**Oligotopic**
	Psammotettix cephalotes	monophagous1	egg	2	macropterous	**Oligotopic**
	Aphrodes makarovi	polyphagous	egg	1	macropterous	**Oligotopic**
	Empoasca decipiens	polyphagous	adult	2	macropterous	Eurytopic
	Graphocraenus ventralis	oligophagous1	egg	1	macropterous	**Oligotopic**
	Acantodelphax spinosa	monophagous2	nymph	1	brachypterous	**Stenotopic**
Species without clear management preferences	*Javesella dubia*	oligophagous1	nymph	2	dimorphic	Eurytopic
	Macrosteles laevis	polyphagous	egg	2	macropterous	Pioneer
	Macrosteles sp.	-	-	-	-	-
	Psammotettix confinis	oligophagous1	egg	2	macropterous	Pioneer
	Laodelphax striatella	polyphagous	nymph	2	dimorphic	Pioneer
	Emelyanoviana mollicula	oligophagous1	egg	3	macropterous	Eurytopic
	Cicadella viridis	polyphagous	egg	2	macropterous	**Oligotopic**

Chapter 3 Determinants shaping community assemblages and species co-occurrence patterns between trophic levels

Reptalus cuspidatus (Fieber, 1876) on *Parthenocissus inserta* in the vineyard floor vegetation (photo: V. Trivellone)

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Abstract

Two or more species coexist if at least two processes have been satisfied: dispersion and habitat (abiotic and biotic) filtering. To highlight mechanisms leading to non-random patterns of species associations, different methodological approaches have been proposed. However, a consensus on the formalized statistical explanation about the influence of processes affecting biological communities has not been reached. Understanding the mechanisms underlying the coexistence between species at different trophic levels may provide deeper insights into assembly processes. In this study, we investigated the relative importance of habitat filtering (environmental and biotic factors) shaping community assemblages by applying a novel multi-analytical approach, i.e., the multiblock redundancy analysis (mbRDA). The analyses were conducted on two model taxa: plants (producers) and leafhoppers (phytophagous), in vineyard agroecosystem in southern Switzerland. Overall mbRDA models explained 51.8% and 54.1% of the variation in plant and leafhopper assemblages, respectively, and the most important blocks of variables were the topography of sampling sites (mainly slope) and the biotic variables. Abiotic filtering processes were relatively more important than biotic ones (plants: 9.6% vs 4.9%; leafhoppers: 14.8% vs 3.8%). However, the total variation in plant and leafhopper communities is more largely explained by the overlap between abiotic and biotic variables (12.5% and 20.5%, respectively), suggesting that biotic relationships are strongly structured by abiotic conditions. Species co-occurrence of plant and leafhopper communities showed a clear evidence of non-randomness segregated patterns. Results of co-occurrence analyses on pairs of polyphagous leafhoppers and common plant species showed a high segregation (40 species pairs out of 57), mainly for plants. This pattern can be reasonably attributed to the net effect of environmental filtering, heterogeneous resource availability and competitive interactions. On the contrary, most common polyphagous leafhoppers showed aggregated patterns (15 species pairs out of 20); suggesting coexistence mechanisms such as host feeding differentiation at local level, different feeding microhabitats on host plant, and similar environmental requirements. Pairwise co-occurrence analyses on monophagous leafhoppers and potential host plants clearly reveal aggregated patterns. Our study revealed a specific role of abiotic factors in shaping communities in vineyards in southern Switzerland, and provided evidences for co-occurrence patterns established in both observed guilds revealing the in-field diversification and trophic interactions.

Keywords biotic and abiotic factors, herbivore, multiblock redundancy analysis, primary producer, trophic level.

Introduction

When two or more species compete in their niche, they probably will coexist if some trade-offs result in differentiation in resources use (MacArthur 1972; Chesson 2000). Reaching a stable species coexistence in terms of abundance can be perturbed by demographic stochasticity due to random dispersal and local extinction (Hubbell 2001). Accordingly, if a group of species coexists in a given spatio-temporal point it means that at least two processes have been concurrently satisfied: the dispersion which has enabled individuals to spread through different habitats and the habitat filtering (both abiotic and biotic) which has permitted populations to persist (Leibold et al. 2004; Siepielski & McPeek 2010; Chase & Myers 2011; Maire et al. 2012). In terrestrial ecosystems, the major habitat filters affecting species assemblage and their coexistence are: topography, land-use, soil type and biotic interaction (Chesson 2000; Pearson & Dawson 2003). Several authors have investigated mechanisms leading to non-random patterns of species associations, and different methods have been proposed to infer the roles of abiotic and biotic drivers (Chalmandrier et al. 2013; Wisz et al. 2013; Blois et al. 2014; Jiang & Ma 2015). Even if not with cause-and-effect evidences, different approaches are very useful to generate hypotheses on the mechanisms underlying the coexistence between species observed at both local, regional and global scales (Chunco, Jobe & Pfennig 2012; Briones-Fourzán 2014). However, the issue concerning the formalized statistical explanation about the relative influence of processes affecting co-occurrence within biological communities has been raised quite often by ecologists (Almeida-Neto et al. 2008; Ulrich, Almeida-Neto & Gotelli 2009; Chase & Myers 2011; Pitta, Giokas & Sfenthourakis 2012; Winegardner et al. 2012; Lin et al. 2014; Veech 2014), and a consensual methodology is still lacking.

The variation observed between biological communities at the regional scale arises from abiotic and biotic stressors, which generate different co-occurrence patterns. Nonetheless, the importance of biotic interactions for shaping broad-scale species distributions has often been neglected or dismissed (Wisz et al. 2013). Also its relative importance with regard to filtering processes is still insufficient (Kraft et al. 2015). One of the key ecological challenges at the moment is to find how to statistically formalize the contribution of biotic interactions, especially when observed species interaction matrices (who interacts with whom) are lacking.

In the present study, we investigated the relative importance of environmental factors and biotic interaction in shaping community assemblages and co-occurrence of species. We considered the major abiotic stressors and two taxa from two trophic levels: primary producers (plants) and phytophagous insects (Hemiptera Auchenorrhyncha; hereafter leafhoppers). Leafhoppers are one of the dominant groups of grassland insect herbivores (Siemann, Tilman & Haarstad 1999). They are sap-feeding insects which feed with their piercing-sucking mouthparts on vascular fluids or on mesophyll tissue (Backus 1988). Despite avoiding a mechanical damage, leafhoppers slow down plant growth with negative consequences on biomass and often transmit diseases (Crawley 1989). Leafhopper assemblages can be characterized based on their degree of dietary specialization, ranging from monophagous species (feeding only on one single plant species) to polyphagous species (feeding on many plant species belonging to different plant families) (Nickel 2003).

In order to better understand the mechanisms behind the plant and leafhoppers assemblages and their relationship with regards to the degree of feeding specialization, the present study aims to answer the following questions: (i) What is the relative importance of biotic and abiotic factors on species assemblages of plants and herbivores? (ii) Are there distinct groups of co-occurring species within and between trophic levels? (iii) What kind of biotic interaction can be inferred from species co-occurring at the local level?

We applied a novel multi-analytical approach to partition the effects of different groups of predictors on the species communities of plants and herbivores, and we provided insights on plant-leafhopper species co-occurrence to infer on their interactions. The study was conducted in 48 vineyards distributed both on flat and terraced areas, thus offering very heterogeneous abiotic and biotic conditions (Trivellone et al. 2014).

Materials and Methods

Study area

The investigation was conducted in vine growing area South of Swiss Alps that is scattered in a region covering almost 3’000 Km2 and including about 1’050 ha of vineyards. Forty-eight vineyard fields were selected based on a design accounting for three abiotic factors (slope, aspect and landscape surrounding) affecting biological community in different way. For a detailed description of the study area and field selection see Trivellone et al. (2014).

Biological sampling

Samplings of plants and leafhoppers were carried out applying a stratified sampling schemes, according to three different homogeneous zones (hereafter sites) detected inside the vineyards: (i) the ground inter-row spacing between grapevines covered by wild vegetation (width ranging from 155 to 185 cm), (ii) the ground row spacing (above grapevines with a standard width of 50 cm) and (iii) the ground slope inter-row spacing (always permanently covered by wild vegetation) (see the scheme in Appendix 3:A1). Overall 68 sampling sites were considered in this study. Each site was sampled with different methods according to taxon type.

Plants - surveys were conducted in June and in August 2011. Species percentage cover of vascular plants was estimated in five 1m x 1m plots randomly distributed over each sampling sites using a decimal scale after Londo (1976). Species nomenclature follows Lauber and Wagner (2009).

Leafhoppers - samplings were carried out in 2011 over eight periods at monthly intervals from March to October. Based on a survey pilot (Trivellone et al. 2012) four complementary sampling techniques were used aiming to effectively intercept most of the occurring species. These techniques are: D-vac suction sampler, beating tray, pitfall traps, and yellow sticky traps (Trivellone et al. 2016). All adults were identified to species level by the first author and preserved in 70% alcohol. Nomenclature followed Ribaut (1936), Ribaut (1952) and Holzinger, Kammerlander and Nickel (2003).

Response variables

The final datasets included 259 vascular plants and 166 leafhoppers species were sampled in 68 sampling sites. Before analyses, plant and leafhopper abundance data were log- and Hellinger-transformed, respectively. All species occurring in less than five sites were removed. The restrictive datasets contained 117 vascular plants and 77 leafhoppers species.

All leafhopper species were classified in two major functional guilds based on degree of dietary specialization (Nickel & Remane 2002): a) species with very narrow food plant spectrum, including monophagous (feeding one single plant species) and oligophagous (feeding on two species from a single genus) species (hereafter specialists), and b) species with a more broad diet, i.e., polyphagous species (hereafter generalists) feeding on more than one genus.

Explanatory variables

We define two different types of variables, abiotic and biotic. The abiotic variables were divided in six thematic data sets (hereafter blocks). Block 1: Management (Man) consists of five variables: the number per year of mowing of wild cover vegetation, application of herbicides, fertilisers (both applied above the vine canopy), insecticides, and fungicide (both applied on vine canopy). Block 2: Topography (Top) consists of five variables: altitude, slope, aspect, solar radiation, and number of solar hours. Block 3: Chemical and physical properties of soil (Soil) consists of nine variables: the amount of organic matter, calcium carbonate, clay, sand, silt, total nitrogen, carbon/nitrogen ratio, inorganic nitrogen, and pH. Block 4: Plant structure of wild cover vegetation (Struc) consists of five variables: cover percentage of grass, moss, bare soil, rock, and litter. Block 5: Landscape composition within 200 m of radius surrounding the sampled sites (Land200) consists of six variables: covered area by vineyards, open vegetated areas, fellows, forests, settlements and water bodies. Block 6: Landscape composition within 500 m of radius (Land500) consists of the same 6 variables as defined above.

In a seventh block we included the biotic variables. These variables were defined as the first two components of a Partial Least-Squares Regression analysis - PLSR (Wold 1966) distinctly performed on the plant and leafhopper community matrices (see details in Statistical approach). The biotic variables are used here as a measure for all possible biotic relationships between the selected trophic levels.

Detailed description of all variables is reported in Appendix 3:A2.

Statistical approach

The data set encompasses one response table and seven blocks (i.e. tables) of explanatory variables. Traditional methods such as redundancy analysis (RDA) are not able to consider the block structure of predictors and are not useable in this case due to the high number of possibly collinear explanatory variables. To solve this problem, we designed a 3-steps statistical framework (Figure 3:1).

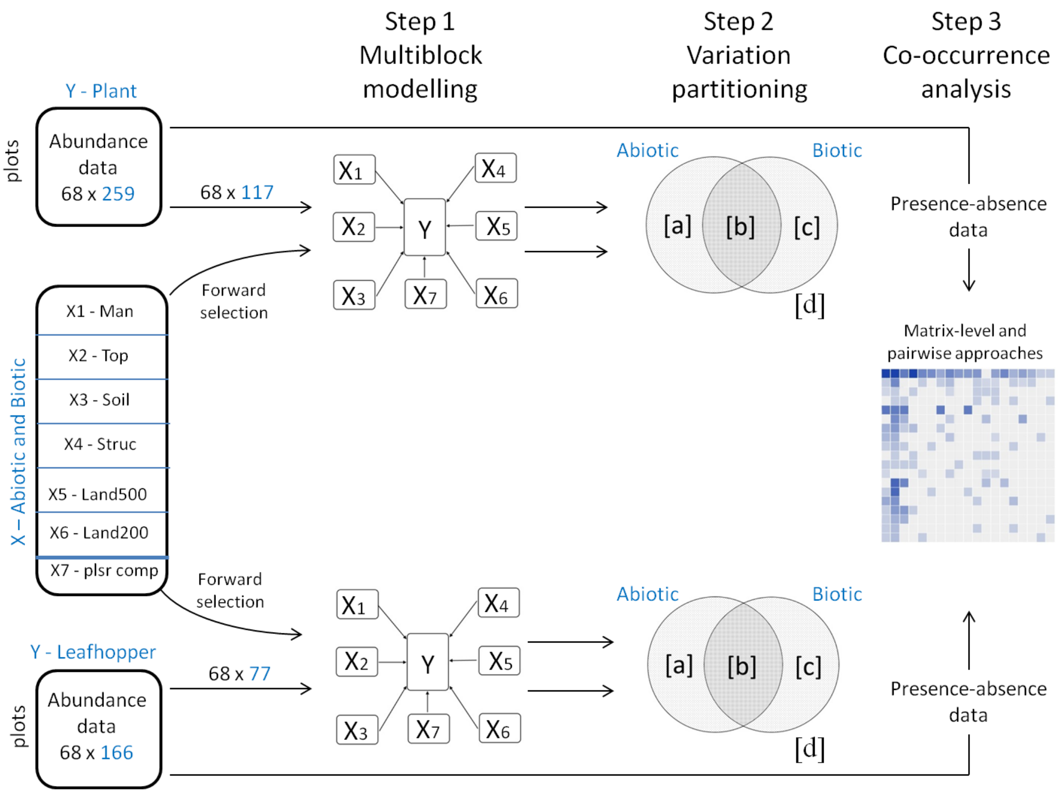

Figure 3:1 Overview of the statistical approach encompassing three steps. X – Abiotic factors, 6 data sets: Man, management; Top, topography; Soil, chemical and physical property of soil; Struc, structure of ground floor vegetation; Land200, landscape composition defined inside a circle of 200 m of radius; Land500, landscape composition defined inside a circle of 500 m of radius around the investigated vineyard; plsr comp, first two PLRS components (or latent vectors) of partial least-squares regression analysis. In Step 2, the total variation of dependent matrix was partitioned in: [a] pure abiotic fraction; [b] pure biotic fraction; [c] shared variance; [d] unexplained variance.

Step 1 - We used the multiblock redundancy analysis (mbRDA) to study the variation in the response variables (Y) that can be explained by the K blocks of explanatory variables (Xi) with i=1, …, K, (Bougeard, Qannari & Rose 2011). The key idea behind this method is that each of the (K+1) tables is summed up with a component, linear combination of the raw variables. Using components instead of raw data allows handling more explanatory variables than in standard analyses and restricts the problem of multicollinearity within explanatory blocks. This is the pivotal principle of orthogonalised regression as components are the best summary of the raw data on the one hand and are orthogonal with each other on the other hand (Massy, 1965).

More precisely, this method derives K components, linear combination of each block of explanatory variables, sought to be as close as possible to a dependent component, linear combination of the response variables. In addition, a global explanatory component related to all the explanatory variables is found as the best summary of the block components. These global explanatory components are used for the regression purpose to avoid integrating too many multicollinear variables. Besides the standard regression coefficients between explanatory and dependent variables, two useful indexes are proposed: (i) the Variable Importance index (VarImp), which allows sorting explanatory variables (P) by order of priority when the number of variables in Y is large, and (ii) the Block Importance index (BlockImp), which assesses the contributions of the explanatory blocks (K) in the overall dependent explanation.

The two models are defined as follow:

$$\mathbf{Y_{plant} \sim Man + Top + Soil + Struc + Land500 + Land200 + Biotic_{leafhopper}} \quad \text{(eqn 1)}$$

$$\mathbf{Y_{leafhopper} \sim Man + Top + Soil + Struc + Land500 + Land200 + Biotic_{plant}} \quad \text{(eqn 2)}$$

where Y_{plant} and $Y_{leafhopper}$ are the restrictive datasets used as response variables in the multiblock models; Man, Top, Soil, Struc, Land200 and Land500 are the six blocks containing the abiotic predictors presented above (Explanatory variables), and $Biotic_{leafhopper}$ and $Biotic_{plant}$ represent the biotic components resulting from the PLSR analysis (see below). For each abiotic block, only significant variables resulted from after the forward selection ($P = 0.05$ after 9999 random permutations) with double-stopping procedure by Blanchet, Legendre and Borcard (2008) (to minimized the problems of the classical forward selection) were included in the analysis. To run mbRDA we used the function *mbpcaiv* in the 'ade4' package (Dray and Dufour 2007) combined with the function *forwards.sel* in 'packfor' package for the forward selection (Dray, Legendre and Blanchet 2007).

Concerning the biotic components ($Biotic_{leafhopper}$ and $Biotic_{plant}$) used in the multiblock models, our aim was to obtain a dimensionally reduced biotic matrix of independent predictor components (or *latent vectors*) which in the meantime represents the biotic relationships between the two trophic levels (plant and leafhopper communities). Hence, we applied PLSR analysis, rather than a non-constrained dimensional reduction technique (e.g. Principal component analysis), because the data values of both independent and dependent variables influence the construction of the *latent vectors* (hereafter PLSR components) used as biotic block. Such as for $Biotic_{leafhopper}$ block, the PLSR model will find the optimal and orthogonal directions in the leafhopper community space that explains the maximum multidimensional variability in the plant space; and vice versa for $Biotic_{plant}$ block. This analysis reduces the number of explanatory variables which is similar to the number of response and also avoids the problem with correlated predictors (Carrascal, Galván & Gordo 2009). The first two vectors of PLSR components are considered for each biotic matrix. The analyses were performed in the package 'pls' implemented in R (R Devolopment Core Team 2010).

Step 2 - Variation partitioning was used to quantify the pure and the shared contribution of abiotic and biotic factors in explaining the variation of plant and leafhopper communities at each sampling site (Borcard, Legendre & Drapeau 1992; Anderson & Cribble 1998; Legendre & Legendre 1998). In this way we aimed at quantifying the portion of the variation explained by the biotic components which are structured by the local abiotic condition. The pure fraction explained by the biotic components is expected to be more close to possible biotic interactions combined with unmeasured environmental factors. Two matrices, i.e. the abiotic matrix containing all significant abiotic variables combined and the biotic matrix with the first two PLRS-components of plants and leafhoppers respectively, were analysed with a series of partial redundancy analyses (pRDA). The pRDA allows the total variation of response variables (plant or leafhopper community) to be partitioned into four fractions: pure abiotic fraction, pure biotic fraction, the portion of biotic variance structured in abiotic fraction (or shared fraction), and the unexplained fraction (Peres-Neto et al. 2006; Borcard, Gillet & Legendre 2011). The variation explained of each fraction was reported as the adjusted coefficient of multiple determination (R^2_{adj}) to take into account of the number of explanatory variables and sample size while preventing the inflation of R^2 values (Peres-Neto et al. 2006). Significance of each source of variation was tested with a Monte Carlo permutation test (999 permutations). The analyses were performed with the *varpart* function in the ‘vegan’ package implemented in R.

Step 3 - The species co-occurrence analysis was performed using two different approaches: (i) matrix-level, and (ii) pairwise (Gotelli 2000; Veech 2014). In both cases we used a presence-absence community matrix from the final datasets of plants and leafhoppers, respectively.

The matrix-level approach was used to describe patterns of species occurrences, and the null hypothesis was that replicated local assemblages were not significantly different from those expected by chance. If this latter is rejected the underlying mechanisms acting on species assemblages might reflect species interaction, environmental filtering or dispersal limitation. To assess the co-occurrence patterns we used the C-score index which quantifies the average number of checkerboard units (i.e. the total number of species that never co-occur in the matrix) calculated for each species pairs (Stone & Roberts 1990), and measures the degree of segregation across sampling sites. The co-occurrence null model randomizes the occurrence matrix based on different kind of constrains (algorithms), in this study the fixed-fixed (FF) algorithm was selected because has a good Type I error rate, and is powerful at detecting patterns in noisy data sets, particularly when used with the C-score (Gotelli & Graves 1996; Gotelli 2000). With FF-algorithm species occurrence totals (rows) and species richness in each site (columns) are preserved, in other words, it retains differences among species in the number of sites they occupy (row sums) and it retains differences among sites in the number of species they harbour (column sums) (Ulrich & Gotelli 2012).

The C-score index was calculated for the empirical matrix and for each calculated matrix by randomization. Lastly, the Standard Effect index of simulated Size (SES) was used to define if the empirical co-occurrence index (C-score) is significantly different from the mean of all simulates indices. SES is calculated as:

$$SES = (I_{obs} - I_{sim}) / Sd_{sim}$$

where I_{obs} is the index calculated on the empirical matrix, I_{sim} is the mean of the indices calculated for each simulated matrix and Sd_{sim} is the standard deviation of all simulated indices. The difference between observed and calculated indices was not significant when SES falls within the range of -2 to 2. For C-score index, SES > 2 indicate significantly non-random species segregation and SES < -2 significantly non-random species aggregation.

The pairwise approach was used to identify which species pairs co-occurred more or less frequently than expected by chance and whether the feeding specialisation of the leafhoppers (monophagous versus polyphagous) showed different patterns. Two sub-matrices of plant-leafhoppers were analysed to characterize the observed patterns: a sub-matrix of 150 rows (62 Polyphagous leafhoppers and 88 most Common plants) by 68 sampling sites (hereafter matrix P-C), and a sub-matrix of 90 rows (56 Monophagous leafhoppers and 34 potential Host plants) by 68 sampling sites (hereafter matrix M-H). In the M-H matrix, the leafhopper species with a specialized feeding behaviour and their plant host were selected according to literature (Nickel & Remane 2002), and if one of them was not recorded in this survey, the congenus species was selected. To test the non-random patterns of co-occurrence we used the FF-algorithm. The C-score for each species pairs was calculated and the significance was detected using confidence limits based on the random distributions (standard CL method). As this method could be affected to a high Type I error, the more restrictive empirical Mean Bayes (Bayes M) method was also applied (Gotelli & Ulrich 2010).

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For the species co-occurrence analyses with the matrix-level approach, the package ‘EcoSimR’ implemented in R (R Development Core Team 2010) was used. The pairwise co-occurrence analyses were performed using PAIRS (Ulrich 2008).

Results

Factors affecting plant communities (Step1)

Among the 36 abiotic variables collected, 16 were significant for the plant community matrix after the forward selection procedure (Appendix 3:A2) and were considered for the first step of analyses. The first two PLSR components of the leafhopper communities explained 31.2% and 13.2% of the variance in the response variable (plant communities), respectively.

The first two dimensions of mbRDA explained 32.1% of the total inertia (respectively 23.4% and 8.7%), 30.2% of the plant community matrix variance and 41.0% of the predictors variance (see Tab. 3:1).

The optimal model (eqn 1) for plant community is obtained by selecting 5 components after a two-fold cross-validation. This model explains 51.8% of the variation in plant communities, 60.2% in Man, 62.1 in Top, 63.8 in Soil, 48.2 in Struc, 54.5 in Land500, 70.5 in Land200 and 88.1 in Biotic.

Table 3:1. Importance of the five first dimensions h and associated cumulated percentages of variance of the datasets explained by the global component t(h) of mbRDA. Abbreviations of explanatory blocks from X1 to X7 in Figure 3:2.

	(h = 1; %)	(h = 2; %)	(h = 3; %)	(h = 4; %)	(h = 5; %)
Y = Plant community					
% of inertia$^{(h)}$	23.4	8.7	5.8	5.5	3.8
Cum % of inertia	23.4	32.1	37.9	43.5	47.2
Cum % of variance of Y expl. by $t^{(1-h)}$	19.7	30.2	37.6	46.5	51.8
Cum % of variance of X expl. by $t^{(1-h)}$	27.2	41.0	50.4	57.5	63.9
Cum % of variance of X_1 expl. by $t^{(1-h)}$	16.2	35.2	42.0	56.3	60.2
Cum % of variance of X_2 expl. by $t^{(1-h)}$	26.9	41.6	53.5	59.2	62.1
Cum % of variance of X_3 expl. by $t^{(1-h)}$	27.7	34.4	43.5	58.4	63.8
Cum % of variance of X_4 expl. by $t^{(1-h)}$	9.0	13.3	24.8	34.2	48.2
Cum % of variance of X_5 expl. by $t^{(1-h)}$	23.8	36.2	40.5	42.0	54.5
Cum % of variance of X_6 expl. by $t^{(1-h)}$	44.2	46.9	65.1	65.1	70.5
Cum % of variance of X_7 expl. by $t^{(1-h)}$	42.7	79.3	83.7	87.2	88.1
Y = Leafhopper community					
% of inertia$^{(h)}$	22.9	12.0	5.8	5.7	4.5
Cum % of inertia	22.9	34.9	40.7	46.3	50.9
Cum % of variance of Y expl. by $t^{(1-h)}$	19.0	32.5	39.8	48.5	54.1
Cum % of variance of X expl. by $t^{(1-h)}$	24.8	40.1	49.3	57.7	64.4
Cum % of variance of X_1 expl. by $t^{(1-h)}$	17.0	33.9	38.6	48.4	54.4
Cum % of variance of X_2 expl. by $t^{(1-h)}$	24.1	36.2	44.7	57.7	60.4
Cum % of variance of X_3 expl. by $t^{(1-h)}$	27.0	37.7	40.9	56.4	57.5
Cum % of variance of X_4 expl. by $t^{(1-h)}$	8.1	9.8	35.0	43.2	70.2
Cum % of variance of X_5 expl. by $t^{(1-h)}$	30.4	42.3	49.3	57.8	61.2
Cum % of variance of X_6 expl. by $t^{(1-h)}$	29.5	47.9	56.8	59.7	62.8
Cum % of variance of X_7 expl. by $t^{(1-h)}$	37.3	72.8	80.1	80.3	84.2

The Block Importance (BlockImp) index was calculated to quantify the contribution of the K=7 explanatory blocks in explaining the variation of plant communities. The threshold value for the block significance is set to 1/K = 0.14 (14.0%). Figure 3:2 shows the weighted cumulated indices over several components included in the model for each blocks. The overall plant communities is mainly driven by topographic (BlockImp = 19.9% $[16.2;23.9]_{95\%}$) and biotic (BlockImp = 24.6% $[24.0;31.0]_{95\%}$) attributes.

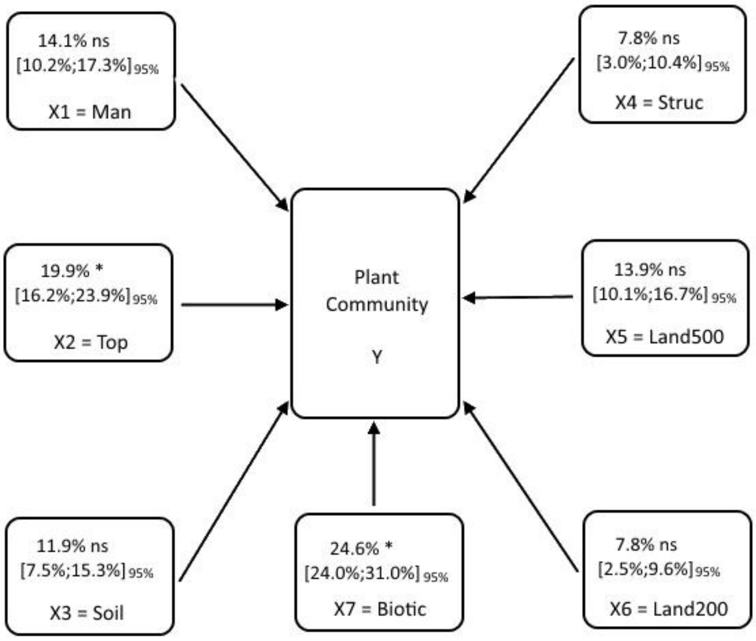
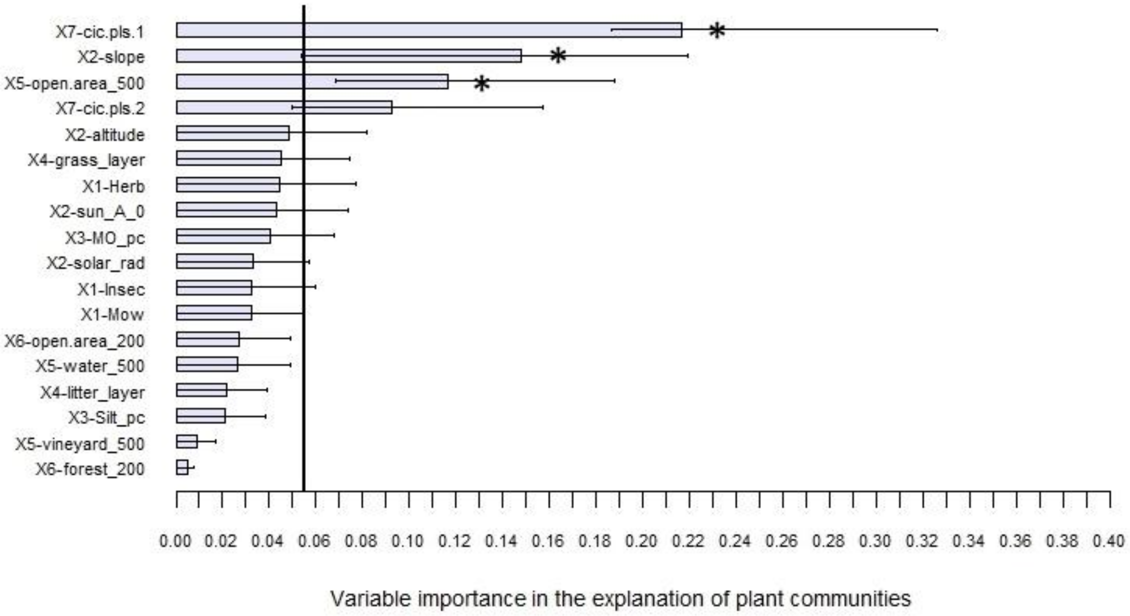


Figure 3:2 Percentage of cumulated contributions, and associated tolerance interval, of each explanatory block (from X1 to X7) in the plant community prediction. The optimal model of mbRDA involving (h=5) components.

Figure 3:3 shows the importance of the single explanatory abiotic and biotic variables on the plant community prediction calculated by means of Variable Importance (VarImp) index with associated standard deviation and tolerance interval. It allows sorting the P=18 abiotic and biotic variables by an overall order of priority. The threshold value for the variable significance is set to 1/P = 0.055 (5.5%). Out of 18, three significant variables affecting plant community were selected: the slope of area (VarImp = 14.7% $[5.4; 21.9]_{95\%}$, X2), the open area surrounding the vineyard up to a 500 m of radius (VarImp = 11.6% [6.8; 18.8]95%, X5) and the first PLSR component of leafhopper community (VarImp = 21.6% $[18.7; 32.6]_{95\%}$, X8).



Figgure 3:3 Contribution of the 18 explanatory variables to the explanation of plant community (Y), based on the Variable Importance index associated with their 95% tolerance interval for a model involving five components. Vertical line is the threshold value (1/P = 0.055), P is the total number of variables in the model. Significant variables (*) are: X7-cic.pls.1 (the first PLSR component of leafhopper community), X2-slope (slope of area), and X5-open area_500 (open area surrounding the vineyard up to a 500 m of radius).

Relative importance of abiotic and biotic variables explaining plant communities (Step2)

The amount of variation accounted by abiotic-biotic shared fraction (R^2_{adj}= 12.5%) was higher than the pure abiotic (R^2_{adj}= 9.6%) and biotic (R^2_{adj}= 4.9%) contributions. This overlap indicates that biotic factors are mainly structured by the environmental characteristics (Fig. 3:4).

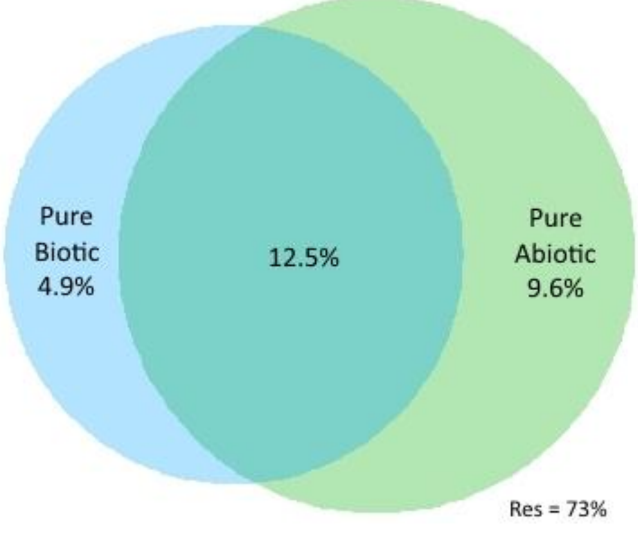


Figure 3:4 Variation partitioning for plant community in each sampling site tested by partial redundancy analyses (pRDA). **Res** provides the unexplained fraction.

Factors affecting leafhopper communities (Step 1)

Among the 36 abiotic variables collected, 18 were significant for the leafhopper community matrix after the forward selection procedure (Appendix 3:A2) and were considered for the first step of analyses. The first two PLSR components of plant communities explained 30.1% and 13.1% of the variance in the response variable (leafhopper communities), respectively.

The first two dimensions of mbRDA explained 34.1% of the total inertia (respectively 22.3% and 11.7%), 31.2% of the leafhopper community matrix variance and 39.8% of the predictors variance (see Tab. 3:1).

The optimal model (eqn 2) for leafhopper community is obtained by selecting 5 components after a two-fold cross-validation. This model explains 54.1% of the variation in Y, 54.4.2% in Man, 60.4 in Top, 57.5 in Soil, 70.2 in Struc, 61.2 in Land500, 62.8 in Land200 and 84.2 in Biotic.

The Block Importance (BlockImp) index was calculated to measure the contribution of the 7 explanatory blocks to explanation of leafhopper community. The threshold value for the block significance is set to 1/K = 0.14 (14.0%). Figure 3:5 shows the weighted cumulated indices over several components included in the model for each blocks. The overall leafhopper communities is driven by topographic (BlockImp = 24.6% $[22.5;29.5]_{95\%}$) and biotic (BlockImp = 20.7% $[17.7;24.9]_{95\%}$) attributes.

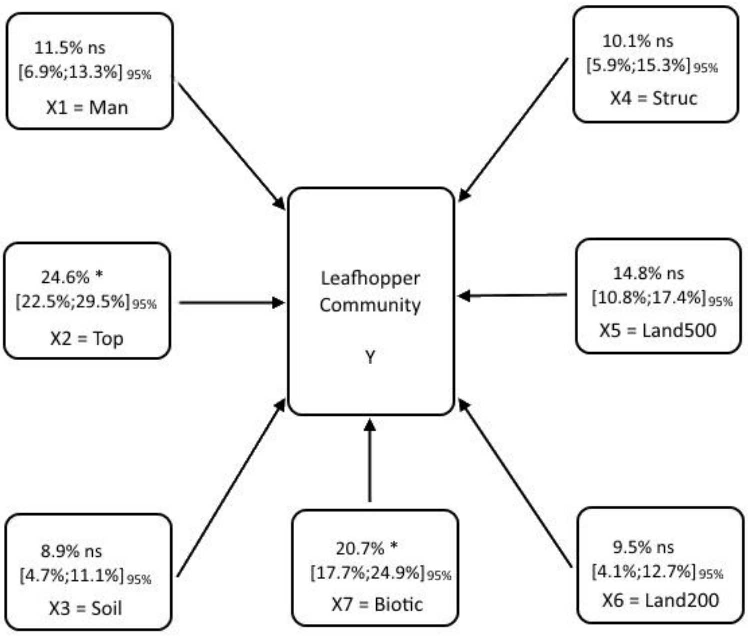


Figure 3:5 Percentage of cumulated contributions, and associated tolerance interval, of each explanatory block (from X1 to X7) in the leafhopper community prediction. For the optimal model of mbRA involving (h=5) components.

Figure 3:6 shows the importance of the single explanatory abiotic and biotic variable on the leafhopper community prediction calculated by means of Variable Importance (VarImp) index with associated standard deviation and tolerance interval. It allows sorting the P=20 abiotic and biotic variables by an overall order of priority. The threshold value for the variable significance is set to 1/P = 0.05 (5.0%). Out of 20, two significant variables affecting leafhopper community were selected: the slope of area (VarImp = 18.5% $[10.8; 28.8]_{95\%}$, X2) and the first PLSR component of plant community (VarImp = 11.4% $[7.8; 17.8]_{95\%}$, X7).

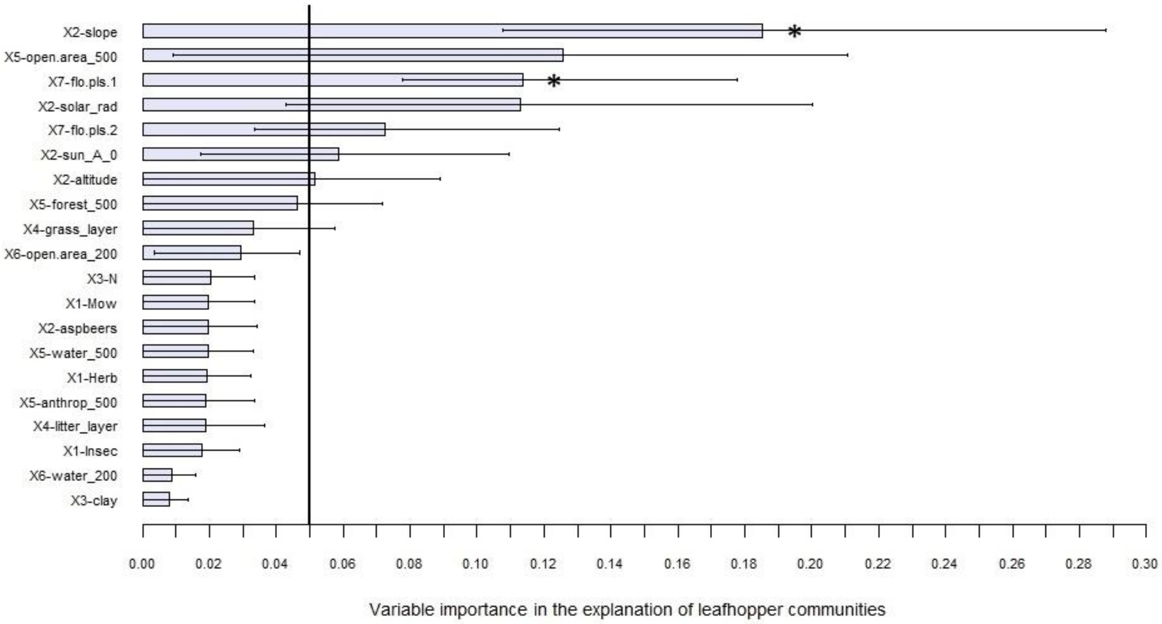


Figure 3:6 Contribution of the 20 explanatory variables to the explanation of leafhopper communities (Y), based on the Variable Importance index associated with their 95% tolerance interval for a model involving five components. Vertical line is the threshold value (1/P = 0.05), P is the total number of variables in the model. Significant variables (*) are: X2-slope (slope of area) and X7-flo.pls.1 (the first PLSR component of plant community).

Relative importance of abiotic and biotic variables explaining leafhopper communities (Step2)

The amount of variation accounted by abiotic-biotic shared fraction (R^2_{adj}= 20.5%) was much larger than the pure abiotic (R^2_{adj}= 14.8%) and biotic (R^2_{adj}= 3.8%) contribution. This overlap indicates that biotic factors are mainly structured by the environmental characteristics (Fig. 3:7).

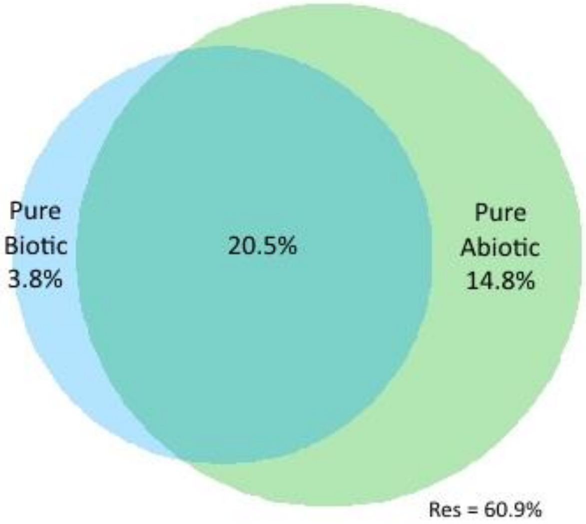


Figure 3:7 Variation partitioning for leafhopper community in each sampling site tested by partial redundancy analyses (pRDA). **Res** provides the unexplained fraction.

<u>Species co-occurrence (Step 3)</u>

The results from null model using the matrix-level approach on plant and leafhopper presence-absence matrices indicated that species co-occurrence is not random in our datasets as confirmed by values of the average Standardized Effect Size (SES). In plant community, C-score index was significantly larger than expected by chance (Average SES = 18.04, p-value < 0.001), indicating a non-random species segregation; in leafhopper community the same trend was observed (Average SES = 8.24, p-value < 0.001).

The pair co-occurrence analysis on the P-C matrix (i.e., Polyphagous leafhoppers and most Common plants) selected a total of 380 significant unique species pairs by CL criterion. Out of these 380 pairs, 56 were further significant based on Mean Bayes criterion (Appendix 3:A3). The C-score indices showed 40 segregated species pairs (five leafhopper-leafhopper ‘l-l’; 12 plant-plant ‘p-p’ and 23 ‘p-l’) and 16 aggregated species pairs (14 ‘l-l’, 0 ‘p-p’ and two ‘p-l’).

The pairs co-occurrence analysis on the M-H matrix (i.e., Monophagous leafhoppers and potential Host plants) selected a total of 133 significant unique species pairs by CL criterion (see step 3 in Statistical approach), 31 of which were also significant based on the Mean Bayes criterion (Appendix 3:A4), all of them are aggregated species pairs (nine ‘l-l’; seven ‘p-p’ and 15 ‘l-p’).

An overview of main results of the three-steps statistical framework are reported in Appendix 3:A5.

Discussions

Relative importance of biotic and abiotic stressors (Question i)

Despite the fact biotic and abiotic constraints are widely assumed to act together to explain the distribution of species and their abundances, many studies usually focus on the effect of abiotic factors alone. In addition, several statistical approaches were tabled to manage all explanatory variables and defined their contribution in shaping the biological communities but none of them got full inside the scientific community (Wisz et al. 2013). In order to consider all major factors affecting plant and leafhopper communities, we used two different statistical approaches which provide complementary information when used together: multi block modelling (mbRDA) and variation partitioning. The mbRDA approach was first originally developed for epidemiological analysis (Bougeard, Qannari & Rose 2011; Bougeard et al. 2012; Bougeard & Cardinal 2014). Interestingly, this method allows to assess the influence of more than four groups of explanatory variables on community assemblages taking into account both the contribution of group of variables (blocks) and single variables. Variation partitioning has a unique characteristic to estimate the shared variation between groups of explanatory variables (4 groups as maximum).

The mbRDA optimal models selected for plant and leafhopper communities (eqn 1 and eqn 2) explained more than half of the variation within the assemblages observed in vineyards in southern Switzerland (51.8% and 54.1%, respectively). The most important variable blocks are topographic and biotic ones, indicating that both abiotic conditions and biotic interaction shape species distributions and assemblages. Moreover, the abiotic variables showed a higher relative importance than the biotic one, in both trophic levels (9.6% vs 4.9% and 14.8% vs 3.8%, respectively) indicating that the abiotic filtering processes are relatively more important than biotic interaction. It is worth noting that the most important abiotic variables were slope of sampling sites and landscape composition (inside a circle of radius 500 m) for plants; and slope of sampling sites for leafhopper communities. Others abiotic factors, such as management, chemo-physical properties of soil and vegetation structure, did not influence communities composition of this two trophic levels in vineyards. The results suggest that the differentiation of communities is due to structural factors of vineyard and/or occur at landscape level. On the other hand, the total variation in plant and leafhopper communities is more largely explained by the overlap (12.5% and 20.5%, respectively) between abiotic and biotic variables which account for an appreciable amounts of variance, suggesting that communities are structured by the interactions between the characteristics of the species available in the regional pool and the environmental conditions, which shape the assemblages at local level.

Groups of co-occurring species pairs (ii) and ecological processes (iii)

Null model test has been successfully applied to terrestrial animal communities with the aim to investigate co-occurrence patterns (Gotelli & Ellison 2002; Fiera & Ulrich 2012; Ingimarsdóttir et al. 2012; Jiménez, Decaëns & Rossi 2012; Lin et al. 2014). Species co-occurrence using the matrix-level approach for plant and leafhopper assemblages in vineyards of southern Switzerland showed a clear evidence of non-randomness patterns where species pairs mainly segregate having significantly less co-occurrence than expected by chance. As highlighted in Gotelli and McCabe (2002) many empirical data sets exhibit segregation pattern but they must contain necessarily some species pairs that are aggregated (Stone & Roberts 1990). Many studies revealed that an overall segregated pattern do not necessarily suggest competition, but could emerge as a result of differentiation in habitat requirement or phylogenetic history (Ulrich & Gotelli 2013); and usually these hypotheses are never mutually exclusive (Ricklefs & Schluter 1993). Ulrich and Gotelli (2012) pointed out some advantages using a fixed-fixed algorithm (F-F) which respects the relative contribution of factors that are not related to species interactions which may influence widespread heterogeneity in species richness and in species occurrences. As the plant and leafhopper assemblages were also significantly affected by topographic characteristics of sampling sites (see results from multi block analyses), choosing a FF algorithm was the best choice. Results of pairwise co-occurrence analyses on Polyphagous leafhoppers-Common plant species (P-C) matrix showed a high frequency of segregated species pairs (40 out of 57) and this was consistent with the co-occurrence pattern of entire communities. Among them, segregated pattern was more important within plant community where all selected species pairs (12) were segregated; and mainly species pairs showed contrasting environmental requirements (e.g. *Veronica arvensis* Linnaeus, usually on dry soils and *Rumex acetosa* Linnaeus, mainly in wet meadows). Observed segregated patterns for plant can be reasonably attributed here to the net effect of environmental filtering, heterogeneous resource availability and competitive interactions, leading to niche differentiation among species, also consistent with widespread evidence reported in Tilman (1982) and Keddy (1989). On the contrary, leafhopper-leafhopper species pairs moved toward an aggregated pattern with 15 species pairs out of 20 pairs in total. These results suggest that most polyphagous and common leafhoppers co-exist in vineyard agroecosystems, probably because host feeding differentiation at local level, different feeding microhabitats on host plant and similar environmental requirements between species which could result in attraction even in the absence of interactions (e.g. facilitation) (Novotny et al. 2012). For instance, *Laodelphax striatella* (Fallén) is aggregated with 12 different leafhopper species, all of them belonging to different families or sub-families and showing different feeding site on the host plant. In our study, the overall segregated observed patterns of leafhoppers can be reasonably attributed to abiotic factors mainly (i.e. slope of sampling sites) as also showed in the first two steps of our analyses. Our findings confirm the hypothesis of a high potential of diversification of leafhopper communities of vineyards in southern Switzerland due to high capability in resource partition within the host plant (i.e. feeding site differentiation).

The pairwise co-occurrence analyses on Monophagous leafhoppers-potential Host plants (M-H) matrix clearly reveal an aggregated pattern. As expected, the majority of species pairs were selected from leafhopper-plant association and species-specific phytophagous-host plant relationships were highlighted. Out of 15 leafhopper-plant species pairs only one pair, *Horvathianella palliceps* (Horvath) and *Chrysopogon gryllus* (Linnaeus), confirms the relationship already reported in literature; whereas another species pair, *Kelisia guttulifera* (Kirschbaum) and *Carex hirta* Linnaeus, confirms previous records at genus level only (Nickel & Remane 2002).

Conclusion and perspectives

Discern between pure environmental filtering (sensu stricto) and biotic interaction (broad sense) with the aim of understanding the processes involved is still a controversial issue (Kraft 2014). Our study focusing on different community patterns observed for two linked trophic levels, revealed a specific role of abiotic factors in shaping communities in vineyards in southern Switzerland. Moreover, we provided evidences for co-occurrence patterns established in both observed guilds revealing the in-field diversification and trophic interactions are the main factors which can cause segregation or aggregation.

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Appendices

Scheme 3:A1 Representation of a "vineyard model" partitioned in 3 homogeneous zones. I flat inter-row spacing between grapevines covered by wild vegetation (width ranging from 155 to 185 cm); R on-row spacing (above grapevines with a standard width of 50 cm) and S sloped inter-row spacing(always permanently covered by wild vegetation).

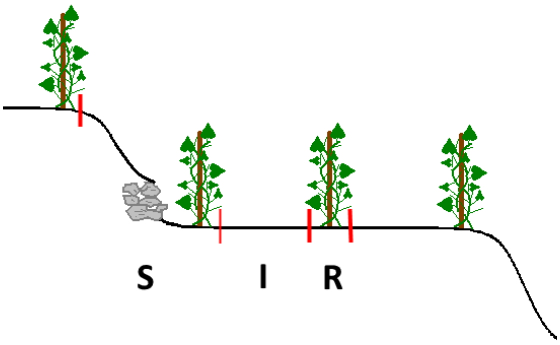

Table 3:A2 Overview of abiotic and biotic variables assigned to each investigated vineyard in 2011. (a) Significant abiotic variables after the forward selection procedure are reported for plant and leafhopper communities. (b) Management variables were collected by administering specific questionnaires to vinegrowers. (c) All physical and chemical analyses were carried out by SolConseil (Changins). (d) Vegetation structure were measured in August. A rectangular of about 800 x 250 m was placed around each pitfall trap station and percentage of each category was recorded. (e) Six landscape cover units have been defined and percentage of each unit was collected by means of georeferenced images and digital cartographic model of Switzerland (resolution of 25 x 25 m, Vector 25 of Swisstopo) based on the topographic 1:25,000 maps, using a geographic information system (ArcGis 10). Data were collected inside a circle of 500 m and 200m of radius around the investigated vineyard.

			Significant variable (a)		
Block	**Variable**	**Unit**	**plant**	**leafhopper**	**Description**
Abiotic variables					
[Man] - Management (b)	Mowing	[n°/year]	Yes	Yes	Number of mowing of ground cover vegetation
	Herbicide	[n°/year]	Yes	Yes	Number of applications of herbicide (organophosphorus compounds and glufosinate, mainly)
	Fertilisers	[n°/year]	-	-	Number of applications of fertiliser (NPK: nitrogen, phosphorus and potassium)
	Insecticide	[n°/year]	Yes	Yes	Number of applications of insecticide (inhibitors of chitin synthesis)
	Fungicide	[n°/year]	-	-	Number of applications of fungicide (different formulations against Powdery Mildew, Downy Mildew and Black Rot)
[Top] - Topography	Aspect	-	-	Yes	Xtr =cos(radianti(X-45°))+1 where X = number degrees
	Slope	[°]	Yes	Yes	Slope of vineyard and of each zone inside

			Significant variable (a)		
Block	**Variable**	**Unit**	**plant**	**leafhopper**	**Description**
	Solar Radiation	[W/m2]	Yes	Yes	Mean of solar radiation in vegetative period (April-October) in 2011
	Solar time	[hours/day]	Yes	Yes	Number of sunlight hours measured on the center of vineyard
	Altitude	[m]	Yes	Yes	Altitude above see level
[Soil] - Chemical and physical property (c)	Hand texture analysis	%	Silt	Clay	Proportion of: clay, silt, and sand
	MO	%	Yes	-	Organic matter content
	CaCO3	%	-	-	Total content of calcium carbonate
	pH	-	-	-	pH of two samples of soil
	Ntot	%	-	Yes	Total nitrogen content
	C/N	-	-	-	Carbon/nitrogen ratio
	Ninorg	[kg/ha]	-	-	Total inorganic nitrogen content
[Struc] - Structure of ground vegetation	Grass	%	Yes	Yes	Categories of vegetation (d)
	Moss	%	-	-	
	Bare soil	%	-	-	
	Rock	%	-	-	
	Litter	%	Yes	Yes	
[Land500] - Landscape	Vineyard	[m2]	Yes	-	Landscape composition based on 6 cover units (e)
	Open area	[m2]	Yes	Yes	
	Fallow	[m2]	-	-	
	Forest	[m2]	-	Yes	
	Settlement	[m2]	-	Yes	
	Water	[m2]	Yes	Yes	
[Land200]- Landscape	Vineyard	[m2]	-	-	Landscape composition based on 6 cover units (e)
	Open area	[m2]	Yes	Yes	
	Fallow	[m2]	-	-	
	Forest	[m2]	Yes	-	
	Settlement	[m2]	-	-	
	Water	[m2]	-	Yes	
Biotic variables					
[Biotic leafhopper] - Biotic	Biotic relationship	-	-	-	first two components (or *latent vectors*) of partial least-squares regression analysis(PLRS), obtained

			Significant variable (a)		
Block	**Variable**	**Unit**	**plant**	**leafhopper**	**Description**
contribution		-	-	-	from plant as response variable and leafhopper communities as explanatory variables.
[Biotic plant] - Biotic contribution	Biotic relationship				first two components (or *latent vectors*) of partial least-squares regression analysis(PLRS), obtained from leafhopper as response variable and plant communities as explanatory variables.

Table 3:A3 Significant plant and leafhopper species pairs selected from the P-C matrix by Mean Bayes criterion in pairs co-occurrence analyses. Pairs of species are ordered by type of species pairs (**Pair**): l-l leafhopper-leafhopper; l-p leafhopper-plant; p-p plant-plant. **Occ1** and Occ2: occurrence of Species 1 and Species 2, respectively.

Pair	**Species 1**	**Occ1**	**Species 2**	**Occ 2**	**Joint Occ**	**P**	**Pattern**
l-l	*Hyal.obsoletus*	43	*Scap.titanus*	38	26	3.22E-06	Segregated
l-l	*Mego.scanicus*	43	*Scap.titanus*	38	25	0.0007	Segregated
l-l	*Phil.spumarius*	39	*Scap.titanus*	38	25	0.0002	Segregated
l-l	*Delt.pulicaris*	37	*Scap.titanus*	38	24	1.43E-06	Segregated
l-l	*Delt.pulicaris*	37	*Rept.cuspidatus*	37	17	0.0000	Segregated
l-p	*Macr.cristatus*	50	*Pla.lanceolata*	36	26	0.0008	Segregated
l-p	*Zygi.rhamni*	43	*Poa.pratensis*	41	26	0.0034	Segregated
l-p	*Zygi.rhamni*	43	*Pla.lanceolata*	36	24	1.8E-07	Segregated
l-p	*Hyal.obsoletus*	43	*Pla.lanceolata*	36	25	1.01E-06	Segregated
l-p	*Hyal.obsoletus*	43	*Hol.lanatus*	37	22	0.0000	Segregated
l-p	*Mego.scanicus*	43	*Ach.millefolium*	38	25	0.0002	Segregated
l-p	*Mego.scanicus*	43	*Hol.lanatus*	37	25	0.0002	Segregated
l-p	*Scap.titanus*	38	*Hol.lanatus*	37	21	0.0000	Segregated
l-p	*Scap.titanus*	38	*Rum.acetosa*	41	25	4.2E-07	Segregated
l-p	*Scap.titanus*	38	*Ste.media*	41	25	1.07E-05	Segregated
l-p	*Scap.titanus*	38	*Arr.elatius*	41	22	0.0000	Segregated
l-p	*Phil.spumarius*	39	*Hol.lanatus*	37	19	0.0000	Segregated
l-p	*Phil.spumarius*	39	*Poa.pratensis*	41	25	0.0017	Segregated
l-p	*Rept.cuspidatus*	37	*Pla.lanceolata*	36	21	0.0000	Segregated
l-p	*Rept.cuspidatus*	37	*Rum.acetosa*	41	25	0.0006	Segregated
l-p	*Rept.cuspidatus*	37	*Ver.persica*	43	22	0.0000	Segregated
l-p	*Rept.cuspidatus*	37	*Cer.fontanum*	38	21	0.0000	Segregated
l-p	*Rept.cuspidatus*	37	*Ver.arvensis*	38	15	0.0000	Segregated
l-p	*Delt.pulicaris*	37	*Con.arvensis*	42	25	0.0007	Segregated
l-p	*Delt.pulicaris*	37	*Poa.pratensis*	41	24	5.48E-06	Segregated
l-p	*Delt.pulicaris*	37	*Cer.fontanum*	38	23	0.0000	Segregated
l-p	*Delt.pulicaris*	37	*Hol.lanatus*	37	16	0.0000	Segregated
l-p	*Delt.pulicaris*	37	*Arr.elatius*	41	14	0.0000	Segregated
p-p	*Dig.sanguinalis*	47	*Rum.acetosa*	41	27	0.0021	Segregated
p-p	*Lol.perenne*	45	*Ver.arvensis*	38	26	0.0011	Segregated
p-p	*Pot.reptans*	45	*Hol.lanatus*	37	25	6.01E-05	Segregated
p-p	*Pot.reptans*	45	*Pla.lanceolata*	36	22	0.0000	Segregated

Pair	Species 1	Occ1	Species 2	Occ 2	Joint Occ	P	Pattern
p-p	*Ver.persica*	43	*Hol.lanatus*	37	18	0.0000	Segregated
p-p	*Con.arvensis*	42	*Rum.acetosa*	41	26	0.0011	Segregated
p-p	*Con.arvensis*	42	*Arr.elatius*	41	26	0.0004	Segregated
p-p	*Ste.media*	41	*Cer.fontanum*	38	25	1.42E-05	Segregated
p-p	*Poa.pratensis*	41	*Pla.lanceolata*	36	25	0.0001	Segregated
p-p	*Rum.acetosa*	41	*Ver.arvensis*	38	22	0.0000	Segregated
p-p	*Cer.fontanum*	38	*Hol.lanatus*	37	19	0.0000	Segregated
p-p	*Hol.lanatus*	37	*Pla.lanceola*	36	24	0.0000	Segregated
l-l	*Laod.striatela*	68	*Zygi.pullula*	67	67	1.85E-06	Agregated
l-l	*Laod.striatela*	68	*Emel.mollicula*	66	66	6.00E-07	Agregated
l-l	*Laod.striatela*	68	*Psam.confinis*	64	64	1.71E-05	Agregated
l-l	*Laod.striatela*	68	*Anac.ribauti*	64	64	2.68E-05	Agregated
l-l	*Laod.striatela*	68	*Eusc.incisus*	63	63	3.73E-05	Agregated
l-l	*Laod.striatela*	68	*Aroc.longiceps*	61	61	5.96E-06	Agregated
l-l	*Laod.striatela*	68	*Aphr.makarovi*	53	53	0.0019	Agregated
l-l	*Laod.striatela*	68	*Empo.pteridis*	52	52	0.0030	Agregated
l-l	*Laod.striatela*	68	*Cica.viridis*	51	51	0.0017	Agregated
l-l	*Laod.striatela*	68	*Empo.vitis*	51	51	0.0012	Agregated
l-l	*Laod.striatela*	68	*Balc.punctata*	51	51	0.0028	Agregated
l-l	*Laod.striatela*	68	*Macr.laevis*	50	50	0.0045	Agregated
l-l	*Zygi.pullula*	67	*Cica.viridis*	51	51	0.0017	Agregated
l-l	*Zygi.pullula*	67	*Balc.punctata*	51	51	0.0008	Agregated
l-p	*Laod.striatela*	68	*Tri.repens*	64	64	5.48E-06	Agregated
l-p	*Laod.striatela*	68	*Tar.officinalis*	62	62	0.0000	Agregated

Table 3:A4 Significant plant and leafhopper species pairs selected from M-H matrix by Bayes M criterion in pairs co-occurrence analyses. Pairs of species are ordered by type of species pairs (**Pair**): l-l leafhopper-leafhopper; l-p leafhopper-plant; p-p plant-plant. **Occ1** and **Occ2**: occurrence of Species 1 and Species 2, respectively. (1) Data after Nickel & Remane 2002; in bold species did not recorded in vineyards of southern Switzerland.

Pair	Species 1	Occ	Species 2	Occ	Join Occ	P	Plant host data from literature (1)
l-l	*Adar.exornatus*	33	*Riba.pungens*	12	11	0.0128	-
l-l	*Adar.exornatus*	33	*Ditr.flavipes*	9	8	0.0556	-
l-l	*Muel.fairmairei*	17	*Eupe.cuspidata*	7	6	0.0030	-
l-l	*Mega.sordidula*	19	*Goni.brevis*	5	4	0.0199	-
l-l	*Sten.major*	13	*Rhop.elongatus*	5	4	0.0070	-
l-l	*Riba.pungens*	12	*Rhop.elongatus*	5	4	0.0030	-
l-l	*Ebar.cognatus*	9	*Eupe.cuspidata*	7	5	0.0004	-
l-l	*Ebar.cognatus*	9	*Riba.collina*	4	3	0.0013	-
l-l	*Acan.spinosa*	4	*Acan.denticauda*	3	2	0.0000	-
l-p	*Adar.exornatus*	33	*Car.caryophyllea*	14	12	0.0159	Brachypodium pinnatum
l-p	*Riba.pungens*	12	*Hol.lanatus*	36	10	0.0532	Brachypodium pinnatum

Pair	Species 1	Occ	Species 2	Occ	Join Occ	P	Plant host data from literature (1)
l-p	*Horv.palliceps*	2	*Chr.gryllus*	1	1	0.0000	Chrysopogon gryllus
l-p	*Keli.praecox*	1	*Chr.gryllus*	1	1	0.0000	**Carex brizoides**
l-p	*Xant.straminea*	12	*Poa.pratensis*	40	11	0.0267	Agrostis capillaris
l-p	*Muel.fairmairei*	17	*Ant.odoratum*	32	14	0.0130	Holcus mollis
l-p	*Ebar.cognatus*	9	*Ant.odoratum*	32	8	0.0086	Festuca spp.
l-p	*Ebar.cognatus*	9	*Car.hirta*	18	7	0.0078	Festuca spp.
l-p	*Ebar.cognatus*	9	*Car.caryophyllea*	14	7	0.0048	Festuca spp.
l-p	*Ebar.cognatus*	9	*Thy.pulegioides*	14	6	0.0052	Festuca spp.
l-p	*Keli.guttulifera*	10	*Car.hirta*	18	8	0.0035	Carex sylvatica
l-p	*Eupe.cuspidata*	7	*Car.caryophyllea*	14	6	0.0012	Festuca rubra
l-p	*Anak.perspicillata*	5	*Bra.pinnatum*	20	4	0.0271	**Carex flacca**
l-p	*Macr.lividus*	1	*Agr.eupatoria*	1	1	0.0000	**Eleocharis palustris**
l-p	*Macr.lividus*	1	*Hol.mollis*	1	1	0.0000	**Eleocharis palustris**
p-p	*Hol.lanatus*	36	*Leo.hispidus*	19	17	0.0060	-
p-p	*Ant.odoratum*	32	*Bra.pinnatum*	20	16	0.0042	-
p-p	*Ant.odoratum*	32	*Thy.pulegioides*	14	11	0.0299	-
p-p	*Leo.hispidus*	19	*Car.caryophyllea*	14	10	0.0046	-
p-p	*Bro.erectus*	3	*Chr.gryllus*	1	1	0.0000	-
p-p	*Agr.eupatoria*	1	*Hol.mollis*	1	1	0.0000	-
p-p	*Fes.arundinacea*	1	*Fes.rubra*	1	1	0.0000	-

Table 3:A5 Overview of results of the 3-steps statistical framework applied to plant and leafhopper communities.

	Type of Output		**Description**	**Plant**	**Leafhopper**	
Step 1	mbRDA optimal model		% variance explained	51.8	54.1	
	Block importance	Topography	% variance explained	19.9	24.6	
		Biotic	% variance explained	24.6	20.7	
	Variable importance	1° PLSR comp	% variance explained	21.6	11.4	
		Slope	% variance explained	14.7	18.5	
		Open area-500	% variance explained	11.6	n.s.	
Step 2	Variation partitioning	Abiotic	% variance explained	9.6	14.8	
		Biotic	% variance explained	4.9	3.8	
		Shared	% variance explained	12.5	20.5	
Step 3	Matrix-level approach (1)			(S)	(S)	
				p-p	**l-l**	**p-l**
	Pairwise approach	P-C matrix	n° significant associations	12 (S)	5 (S); 14 (A)	23 (S) 2 (A)
		M-H matrix	n° significant associations	7 (A)	9 (A)	15 (A)

(1) P-C matrix= Polyphagous leafhoppers-Common plant species matrix; M-H matrix= Monophagous leafhoppers-potential Host plants matrix; S= segregation; A= aggregation; p-p= plant-plant; l-l= leafhopper-leafhopper; p-l= plant-leafhopper.

Chapter 4 Indicators for taxonomic and functional aspects of biodiversity in the vineyard agroecosystem of Southern Switzerland

Vegetation of a south-facing vineyard in Rovio (photo: V. Trivellone)

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Abstract

It is widely accepted that the concept of biodiversity embraces two essential and complementary components: taxonomic and functional diversity. Our goal is to produce a list of plant species predictive of high taxonomic and functional biodiversity values and discuss their use within biodiversity monitoring programmes. We selected a representative sample of 48 vineyard areas from Southern Switzerland, and vegetation from the ground cover was sampled from within a total of 120 sampling plots. We considered ten widely used functional traits and selected six taxonomic and functional indices. We applied a two-step analysis: (i) using Threshold Indicator Taxa Analysis (TITAN) based on the above mentioned biodiversity indices, we defined 3 groups of sampling plots with low (**L**), medium (**M**) and high (**H**) biodiversity values; (ii) using the Indicator Value analysis, we identify indicator species that are significantly associated with the above-mentioned groups and their combinations. In total, 259 vascular plants were identified across the sampling plots. As a whole, 52 species were significant indicators for groups with high and mid-to-high biodiversity values. Out of all indicator species, 24 (46%) were exclusively selected by functional biodiversity indices whereas only 10 (19%) were associated with taxonomic indices. Eighteen (35% of the total) species were selected by both types of indices. We point out that indicator species associated with two different aspects of biodiversity show a high degree of complementarity. Our results emphasize the need to consider functional aspects of biodiversity in diversity-conservation strategies when the objectives are to preserve both taxonomic diversity and ecosystem functioning.

Keywords biodiversity surrogates, functional indices, indicator species, Switzerland, vineyard floor vegetation.

Introduction

There is general agreement that agricultural intensification has a deep impact on biodiversity with possible cascade effects on ecosystem functions and service delivery (Millennium Ecosystem Assessment, 2005; Moonen and Bàrberi, 2008). The synergy of conservation efforts and sustainable production can be achieved by designing well-drafted and targeted agri-environmental strategies (Tscharntke *et al*., 2012). Selecting reliable indicators is the crucial step in assessing the effectiveness of agri-environmental schemes with respect to biodiversity conservation and its associated services (Noss, 1990; Mace and Baillie, 2007; Teder *et al*., 2007; de Bello *et al*., 2010). *Indicators* are organisms or attributes of communities which can be used to provide information on biodiversity status and trends (Teder *et al*., 2007).

Biodiversity can be measured in many different ways. Among these, taxonomic diversity and functional diversity are two essential and complementary components (Lyashevska and Farnsworth, 2012). Taxonomic diversity expresses the variety of species in a community. Functional diversity represents the value and range of functional traits in a community and its relation to related ecosystem functionality (Diaz *et al*., 2007). Some authors have highlighted that an ecosystem can be inhabited by many species, and thus reveals high species richness, while showing low functional diversity if species share the same type of traits (Gerisch *et al*., 2012; Moretti *et al*., 2009). Despite increasing research aiming to assess these components of biodiversity (e.g. Hodgson *et al*., 2005; Devictor *et al*., 2010; Cadotte *et al*., 2011; Sattler *et al*., in press), functional diversity is still scarcely included in biodiversity monitoring programmes (Woodwell, 2002; Vandewalle *et al*., 2010; Perrings *et al*., 2011).

We assess the use of different indicator species for monitoring taxonomic and functional diversity using vineyards as a model system. European vineyards are often home to a wide range of plants, sometimes perceived as weeds (Lososová *et al*., 2003), which inhabit different portions of the vineyard, such as below the grapevine, in the inter-space between rows and on vegetated slopes, or in terraced vineyards only when the latter are present. The type and pressure of management practices in vineyards strongly determine the vegetation structure of these habitats. Indeed, anthropogenic disturbance has been indicated as one of the main driving forces controlling both functional and taxonomic aspects of biodiversity in vineyards (Bruggisser *et al*., 2010; Trivellone *et al*., 2012). In Swiss vineyards, ecological direct payments (subsidies) to promote a high level of biodiversity are only granted to vine-growers that satisfy a number of ecological requirements (Swiss Federal Ordinance on Direct Payments in Agriculture, OPD of 23 October 2013). Basically, a quality value for the vineyard is calculated by a monitoring scheme using a scored list of 59 non-productive plants belonging to the Red List or species of particular interest.

Our aim was to identify a list of plant species predictive of high taxonomic and functional biodiversity values. We then discuss how the selected species can be integrated for practical implementation in a monitoring scheme for the payment of subsidies to Swiss vineyards. As a case study, we selected a representative sample of vineyard areas from the Southern Alpine region of Switzerland.

Material and Methods

Study area and experimental design

The study was conducted in 48 vineyards (hereafter referred to as study sites) distributed across the main vine growing area in Southern Switzerland (Appendix, Fig. 4:A1), from Ludiano (46°25’N–8°58’E) to Pedrinate (45°49’N–9°00’E), the Northernmost and Southernmost sites, respectively, ranging from 199 m to 589 m a.s.l. The area is characterized by a moist warm-temperate climate and mean annual precipitation ranging from 1 600 mm (South) to 1 700 mm (North), and mean monthly temperatures ranging from 0.5 °C (North) to 1.6 °C (South) in January and from 21.2 °C (North) to 23.5 °C (South) in July (Spinedi and Isotta, 2004).

The 48 study sites were selected using a design that accounted for the three main variables characterizing the vineyard agroecosystem in the study region, i.e. aspect (24 sites were exposed

SE-SW; 24 sites NE-NW), slope (24 sites were on a plain: <5°; 24 sites were terraced >10°) and the dominant landscape element (>50% cover) surrounding the vineyard within a radius of 500 m (16 sites were dominated by forest, 16 sites by settlements, 16 sites by open areas). Topography and landscape data were obtained using a 25 m cell size digital elevation model (DHM25©2004) and a swiss map in scale 1:25'000 in vector format (VECTOR25), both provided by Swisstopo and implemented with ArcGis 10 (ESRI, 2011). In this way, we obtained a full balanced design with the 48 study sites grouped among the three groups of variables as detailed in Appendix (Table 4:A1).

Vegetation sampling

Vegetation was sampled at each study site during two distinct sampling periods (June and August), in order to include plant species with early and late phenology. Five 1m × 1m quadrats were randomly chosen in each of the different habitats present within each vineyard: 2 habitats-on-plain, i.e. below the grapevine’s rows (Row-on-plain) and on the inter-space between rows (Interrow-on-plain) and 3 habitats-on-terrace, i.e. on vegetated slopes (Slope-on-terrace) and the same habitats as on the plain but in terraced vineyards (Row-on-terrace, Interrow-on-terrace).We thus surveyed a total of 1 200 quadrats (48 study sites x 5 habitats x 5 replicates). All vascular plant species rooting within each quadrat were identified and the percentage cover of each species was estimated using a decimal scale after Londo (1976). Cover of bare soil and rocks was also taken into account. Species nomenclature follows Lauber and Wagner (2009).

Functional traits selection

We considered ten widely used morphological and phenological characteristics of plants as functional traits, *sensu* Violle *et al*. (2007): plant (vegetative) height (PH), specific leaf area (SLA), leaf dry matter content (LDMC), dispersal syndrome (DS), and seed mass (Sm), obtained from the TRY database (Kattge *et al*., 2011), and growth forms (GF), root depth (RD), reserve (or storage) organs (RO), range of flowering (rF), and seed longevity (Sl), taken from Landolt *et al*. (2010) (Table 4:1). We specifically selected traits that determine species' response to both environmental conditions and management (Lavorel and Garnier, 2002; Cornelissen *et al*., 2003).

Taxonomic and functional indices

In order to take taxonomic and functional components of biodiversity into account, we selected six distinct widely used indices. Taxonomic biodiversity was quantified using Species Richness (Ric), Simpson (Sim) and Shannon (Sha) indices (Magurran, 2004), while functional aspects of biodiversity were quantified using Functional Richness (FRic), Functional Divergence (FDiv) (Villéger *et al*., 2008) and Rao’s quadratic entropy (Rao) (Botta-Dukát, 2005). FRic indicates the extent of trait space occupied by a community. This was calculated based on a principal coordinates analysis from a Gower-distance matrix of pairwise distances between species in trait space. FRic was measured as the volume of a convex hull enclosing the principal coordinates of the species present in each community. Contrary to FRic, FDiv index takes the relative abundances of the species into account and it is related to how abundance is distributed within the volume of functional trait space occupied by species. Since the Rao index is the sum of trait dissimilarity among all possible pairs of species, weighted by the product of their relative abundance, it therefore includes information about the evenness of the distribution of functional traits within a community. All indices were calculated with R 2.15.1 (R Development Core Team, 2012) using all species. Functional indices were obtained using the *dbFD* function of the FD package (Laliberté and Legendre, 2010).

Table 4:1 Median values and ranges for 10 functional traits of plants detected in the study.

Functional trait	**Trait code**	**Type**	**Unit**	**Minimum**	**Median**	**Maximum**	**Nr. NA entries**
Growth forms	GF	nominal	10 Levels	1.00	3.00	8.00	0
Plant (vegetative) height	PH	continuous	(m)	0.05	0.37	40.0	0
Specific leaf area	SLA	continuous	(mm^2 mg^{-1})	6.28	24.8	60.8	32
Leaf dry matter content	LDMC	continuous	(g/g)	0.03	0.20	0.45	48
Root depth[a]	RD	ordinal	(cm)	1.00	2.50	5.00	17
Reserve (or storage) organs[b]	RO	nominal	11 Levels	0.00	1.00	1.00	0
Dispersal syndrome[c]	DS	nominale	3 Levels	0.00	0.33	1.00	86
Range of Flowering	rF	continuous	Months	1.00	3.00	12.0	0
Seed longevity	Sl	ordinal	Years	2.00	4.00	5.00	113
Diaspores mass[d]	Sm	continuous	(mg)	0.00	0.95	3487	9

[a] Data was ordered in a meaningful sequence from 1 to 5 ranging root depth values in 9 categories from <25cm to > 200cm.
[b] The dummy variable 0–1 indicates absence or presence of reserve/storage organs.
[c] Fuzzy coded variable.
[d] For Pteridophytes, a factitious value for mass of meiospore was assigned.

Statistical analysis

For each study site, we combined the species data of the five quadrats per *habitat* (Row-on-plain and -on-terrace, Interrow-on-plain and -on-terrace, Slope-on-terrace) over the two sampling periods, for a total of 120 sampling plots (i.e., 24 study sites x 2 habitats-on-plain + 24 study sites x 3 habitats-on-terrace) over the 48 study sites.

We applied a two-step analysis. In the first step, we defined groups of sampling plots with more similar values for the above mentioned biodiversity indices (see *Taxonomic and functional indices* Section). Then using the Threshold Indicator Taxa ANalysis (TITAN) approach (Baker and King, 2010), we detected and quantified community thresholds (sumz+ and sumz-) with regards to "biodiversity gradients" for each diversity index selected in our study. Sampling plots therefore fell into three groups: **L** (low), which indicates sampling plots with biodiversity values lower than the sumz- threshold, **H** (high) for those with biodiversity values higher than the sumz+ threshold and **M** (medium) those with biodiversity values between the sumz- and sumz+ thresholds. This analysis was performed for each diversity index considered in this study (more details about the TITAN analysis are given in Appendix 4:A2). In the second step, we used the Indicator Value analysis (Dufrêne and Legendre, 1997) to identify plant species (hereafter indicator species) significantly associated with the above-mentioned groups and their combinations. The association of species to the sampling plot group was assessed by the indicator value index (IndVal) and its significance (p-value<0.05) was obtained by a randomization procedure (999 permutations) and Holm correction for multiple tests. The index is the product of two components *A* (specificity) and *B* (fidelity), where the former is the probability that a new studied sampling plot belongs to the group associated with the recorded indicator species, and the latter is the probability of finding the species in sampling plots belonging to the group. IndVal index ranges from 0 to 1 and reaches the maximum when all individuals of a species are found in a single group (high fidelity) and when the species occurs in all sampling plots in that group (high specificity). All significant indicator species with a *B* value < 0.25 were removed to discard indicators that occur too rarely (i.e. in less than 25% of sampling plots) as suggested by De Cáceres *et al*. (2012). For each of the six biodiversity indices, only plant species associated to **H** and combined **M**+**H** groups were considered as indicator species of ‘high’ and ‘mid-to-high’ taxonomic or functional biodiversity. The plant species cover percentage values were log-transformed in order to reduce the influence of highly variable taxa on the Indicator Value calculations as recomended by Baker and King (2010). Finally, to assess the degree of complementarity for each indicator species, we used the principal component analysis (PCA) based on the biodiversity indices that define a space of six dimensions and a PCA-plot to visualize the results. Two species positioned far apart on the PCA-plot are considered complementary, whereas species clustered in the multidimensional space are considered more similar in terms of represented biodiversity indices.

Statistical analyses were performed using R 2.15.1 (R Development Core Team, 2012). The Threshold Indicator Taxa ANalysis was run with the package TITAN (Baker and King, 2010). The Indicator Value analysis (IndVal) complemented by the multi-levels pattern analysis was performed using a “multipatt” routine in the “indicspecies” package (De Cáceres and Legendre, 2009; De Cáceres *et al*., 2010).

Results

A total of 259 vascular plants were identified across the 120 sampling plots (Appendix 4:A3). The two community thresholds for each biodiversity index were detected by TITAN (Fig. 4:1 and Appendix 4:A4 for details). Based on these values, three balanced groups of sampling plots were obtained. As a whole, for the six biodiversity indices considered, 52 species were significant indicators for groups **H** and **M** + **H** with a fidelity value >0.25, and they accounted for 20% of the total number of species identified. Depending on the index used, between 9 and 21 indicator species were selected: Ric: 19 (= 37% of the total 52 indicators), Sim: 21 (40%), Sha: 20 (38%), FRic: 24 (46%), FDiv: 11 (21%) and RaoQ: 9 (17%) (Appendix 4:A5). Three indicator species (*Gallium mollugo*, *Erigeron annuus* and *Arrhenatherum elatius*) reached the highest IndVal values for taxonomic indices (Ric, Sim and Sha) as well as for functional richness. For FRic, the IndVal analysis identified *G. mollugo*, *E. annuus* and *A. elatius* as being associated with the combination of groups **M** and **H** (IndVal = 0.810, 0.782 and 0.763, respectively). These high IndVal scores were due to high specificity rather than fidelity. Similarly, although the species associated to the H group only had moderate IndVal values (< 0.589), their specificity was high (0.638-0.934). Two indicator species for high FDiv values, *Taraxacum officinale* and *Veronica persic*a, showed high IndVal scores (0.877 and 0.716, respectively), the former due to high fidelity (*B* = 0.881) and the latter due to high specificity (*A* = 0.739). In addition, both were associated to the **M** + **H** group only. This was also the case with indicator species for high Rao values, with *V. persica*, *Geranium molle*, *Stellaria media* and *Digitaria sanguinalis* being associated with **M** + **H** group. Figure 4:2 shows an overview of specificity and fidelity values for indicator species associated with each of the biodiversity indices. Overall, specificity values range from 0.481 to 0.989 (mean=0.677) and fidelity values from 0.200 to 0.937 (mean= 0.413).

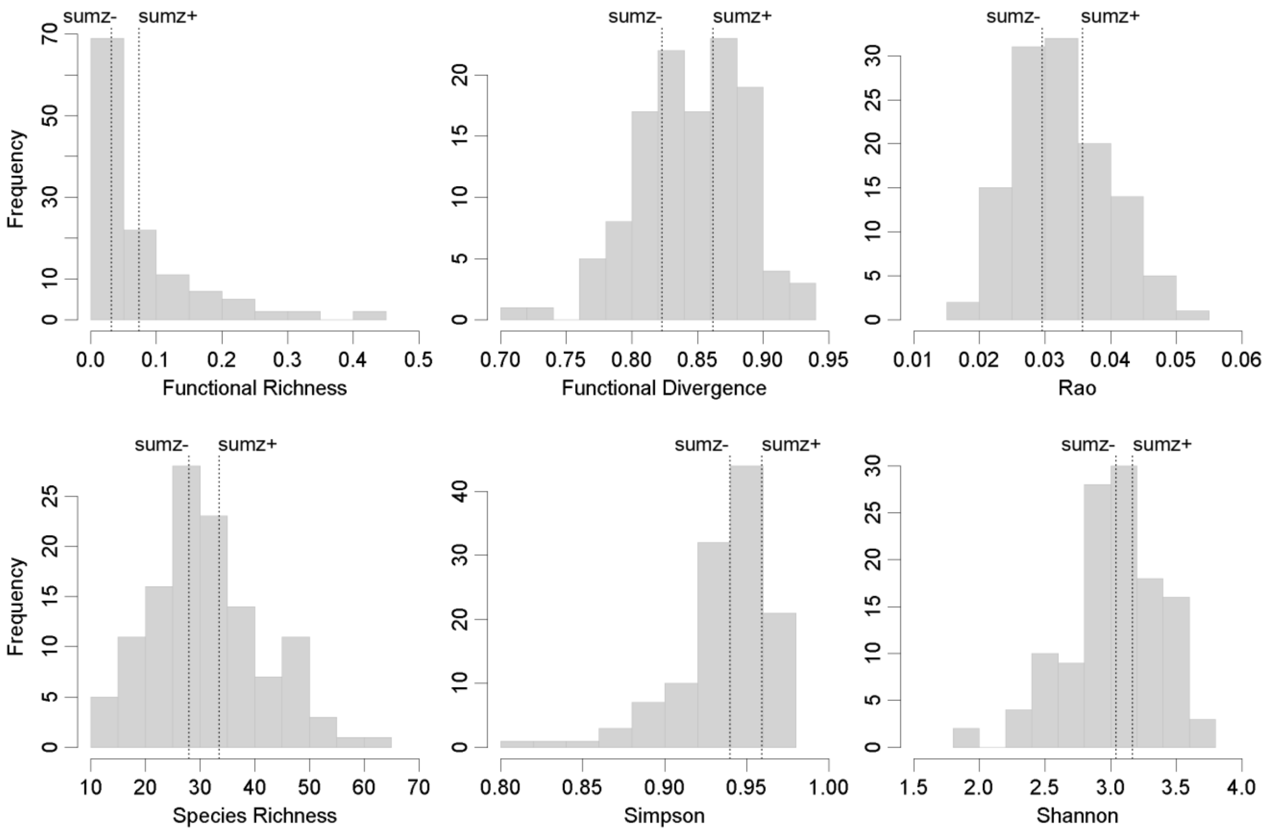

Figure 4 :1 Frequency distributions of the values of each biodiversity index for 120 sampling plots. Dotted lines represent the community threshold values (sum- and sum+) detected by Threshold Indicator Taxa ANalysis (TITAN).

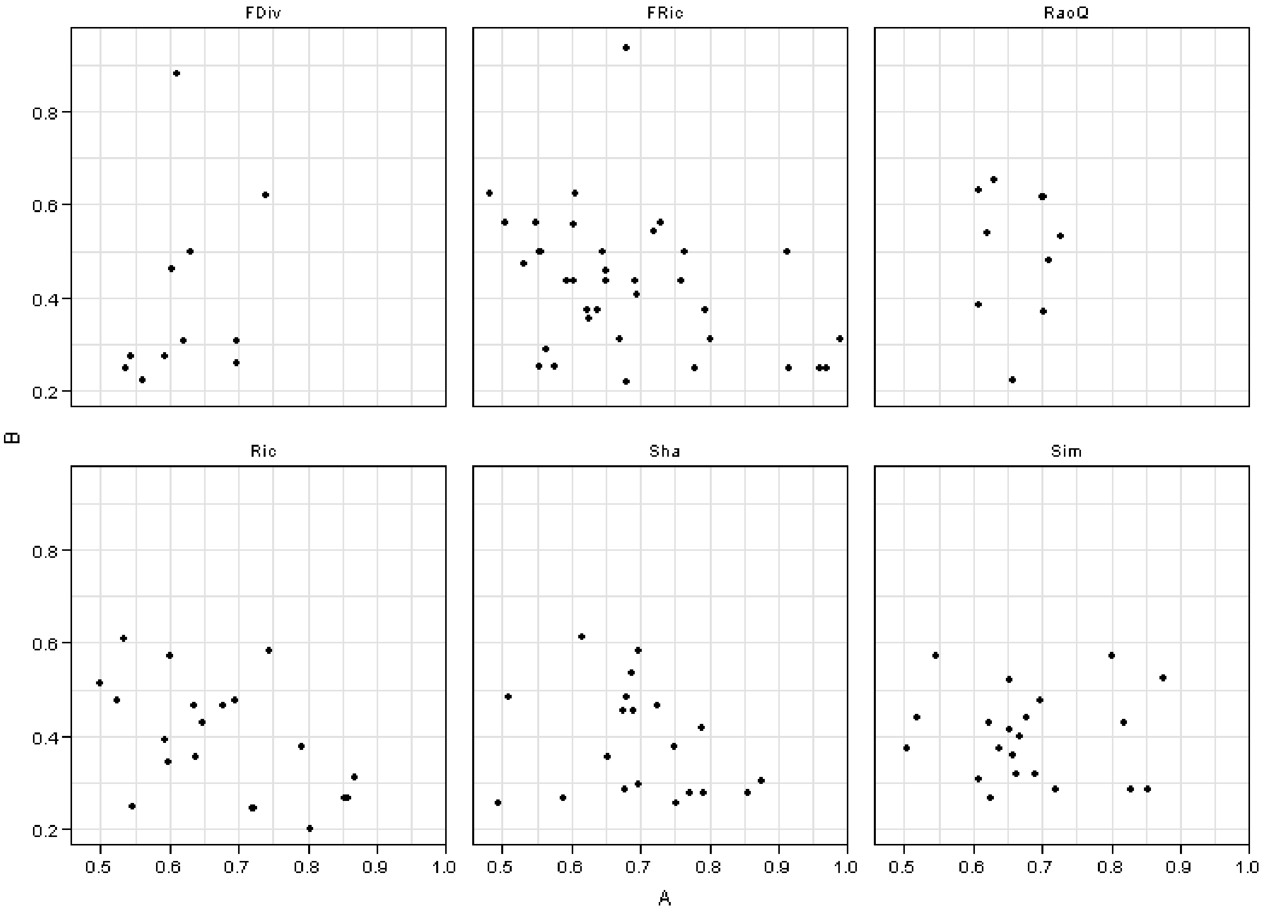

Figure 4:2 Biplots of the specificity (*A*) versus specificity (*B*) values of the indicator species selected by Indicator Value analysis for the 6 biodiversity indices considered (Functional Divergence –Fdiv, Functional Richness –Fric; Rao's quadratic entropy –Rao; Species Richness –Ric; Shannon index – Sha; Simpson index –Sim). Only species associated to sampling plot groups **H** and **M** + **H** were plotted.

Degree of complementarity

Out of 52 indicator species, 24 (46%) species were exclusively selected by functional biodiversity indices whereas only 10 (19%) species were only associated with taxonomic diversity indices. Eighteen (35% of the total) species were selected by both categories of indices: *Achillea millefolium*, *Anthoxanthum odoratum*, *A. elatius*, *Crepis capillaris*, *Cruciata glabra*, *E. annuus*, *G. mollugo*, *Holcus lanatus*, *Rubus fruticosus*, *Silene vulgaris*, *Daucus carota*, *Hypochaeris radicata*, *Oxalis stricta*, *Urtica dioica*, *Artemisia verlotiorum*, *Lotus corniculatus*, *Sanguisorba minor*, and *Silene pratensis*. However, 6, 9 and 7 species were exclusively selected based on FRic, FDiv and Rao, respectively; whilst 1, 3 and 1 species were exclusively selected based on Ric, Sim and Sha, respectively (Appendix 4:A6).

The PCA-biplot (Fig. 4:3) showed that functional (FRic, FDiv and Rao) and taxonomic (Ric, Sim, Sha) biodiversity indices were not correlated. While the three taxonomic indices were projected close to each other, this was not the case for the functional indices. In particular, Rao and FDiv were clearly projected far from the other indices on axis 1 and FRic was separated from the taxonomic indices on axis 2. Two indicator species clustered on the negative side of the PCA axis 1 indicated high FDiv and Rao, whereas at the positive end of PCA axis 1, a cluster of eleven species were associated to high values in the taxonomic biodiversity indices. Two more scattered groups of indicator species were associated to Sim and FRic.

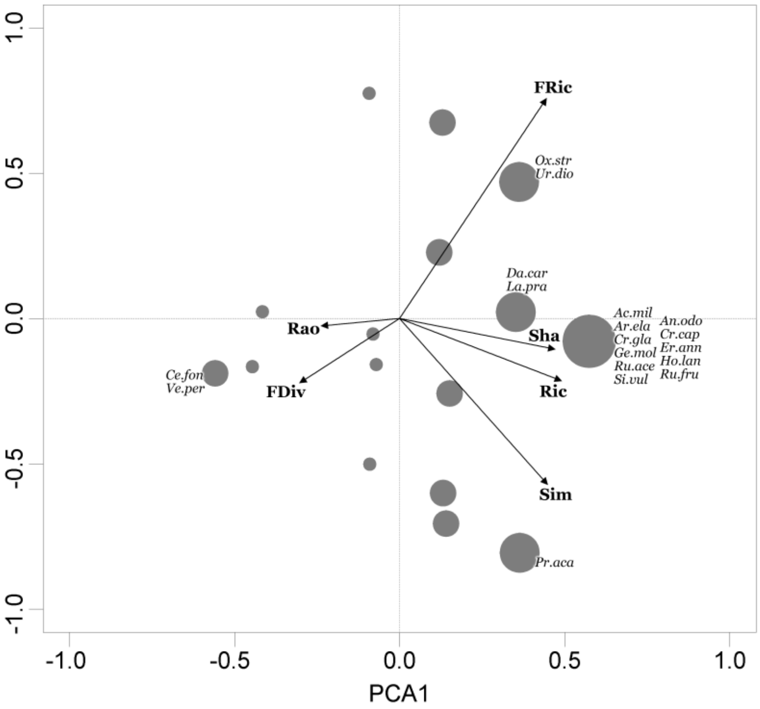

Figure 4:3 Biplot of the Principal Component Analysis (PCA) of selected indicator species (grey dots) and their association with the 6 biodiversity measures (arrows). Only the species most correlated to the first two canonical axes (n = 18 out of 52) are shown. 1st axis explains 54.5%, 2nd 13.3% of the information. *Ac.mil: Achillea millefolium; An.odo: Anthoxanthum odoratum; Ar.ela.: Arrhenatherum elatius; Ce.fon: Cerastium fontanum; Cr.cap: Crepis capillaris; Cr.gla: Cruciata glabra, Da.car: Daucus carota; Er.ann: Erigeron annuus; Ge.mol: Geranium molle; Ho.lan: Holcus lanatus; La.pra: Lathyrus pratensis; Ox.str: Oxalis stricta; Pr.aca: Primula acaulis; Ru.ace: Rumex acetosella; Ru.fru: Rubus fruticosus; Si.vul: Silene vulgaris; Ve.per: Veronica persica; Ur.dio: Urtica dioica.*

Discussion

This study has highlighted how integrating more than one aspect of biodiversity permits the identification of complementary indicator species to cover different components of diversity. Of the 52 indicator species associated with high and mid-to-high values of taxonomic and functional biodiversity, 10 species were exclusive indicators of taxonomic indices, 24 of functional indices, and 18 of both. Functional divergence and Rao's quadratic entropy indices significantly selected the largest group of indicator species which were associated to the functional biodiversity aspect only and, at the same time, showed high complementarity towards the Functional Richness index and the three taxonomic biodiversity indices (i.e. Species Richness, Simpson and Shannon indices). This study has also shown that multiple indicator species are required to monitor diversity in general and especially functional diversity.

Given the multidimensional nature of biodiversity, selecting an optimal set of *indicators* of overall biodiversity is of crucial importance – and can indeed be considered the holy grail of biodiversity management. Several authors have addressed this topic based on simulated community data, e.g. Lyashevska and Farnsworth (2012) highlighted that species richness missed 88.6% of the total diversity, emphasising the importance of considering other biodiversity aspects as well. According to Sattler *et al*. (in press), the selection of umbrella species (indicator species) associated with multiple biodiversity facets provide a useful tool to promote urban biodiversity in central Europe. In our results, eighteen species were associated to both biodiversity aspects. However, it is worth noting that only Functional Richness was weakly associated with the three taxonomic biodiversity indices (Fig. 4:3) as already shown by several authors (e.g. Cornwell *et al*., 2006; Pakeman, 2011). We believe that taxonomic diversity is correlated to functional diversity in terms of the range of traits. The fact that FDiv and Rao represent complementary components of functional diversity implies that indicator species corresponding to these indices should be included in biodiversity monitoring protocols. In an empirical investigation of a river floodplain, Gallardo *et al*. (2011) demonstrated that a combination of measures (i.e. functional diversity, size diversity and taxonomic distinctness) were useful in assessing environmental changes and determined their utility as relevant *indicators* of ecosystem biodiversity and functionality. From a conservation point of view, priorities and strategies are thus slowly moving towards a more integrated approach (Devictor *et al*., 2010, Villéger *et al*., 2010).

Characterisation of the indicator species

Species identified by the Indicator Value analysis as being indicators of diversity in vineyards, typically belong to vegetation types such as low-altitude mown grasslands, dry grasslands, mesophilous forests, nutrient-poor edge habitats, or ruderal areas (Delarze and Gonseth, 2008). Unsurprisingly, a large proportion (17 in total) of species indicative of high and moderately high biodiversity, are characteristic of hay-meadows on moderately nutrient-rich, relatively moist soils (such as *A. millefolium*, *A. elatius*, or *S. vulgaris*), and are resistant to a moderate cutting regime (up to two cuts per year, e.g. *A. odoratum*, *Cerastium fontanum*), corresponding to the typical vegetation of southern Swiss vineyards. The list also includes competitive-ruderal species (CR species *sensu* Grime, 2001), which take advantage of vegetation gaps due to their ability to spread quickly by vegetative growth after disturbance (e.g. *Poa trivialis*). Seven plant indicator species in our list (e.g. *Carex caryophyllea*, *D. carota* or *Brachypodium pinnatum*) are frequently dominant in semi-dry grasslands. These species are considered to be vulnerable to mowing (Briemle and Ellenberg, 1994). Furthermore, under a moderate mowing regime they can take up soil N and thus represent efficient N sinks to help keep the soil relatively nutrient-poor. Two species characteristic of more shady habitats (*Primula acaulis* and *Hedera helix*) were mainly recorded on vegetated slopes in terraced vineyards with a lower solar incidence. Amongst others, the indicator species *C. glabra* or Veronica chamaedrys are typical of nutrient poor edge habitats, occurring in structure-rich vineyards. Finally, an important group (13) of indicator species is ruderal annual and perennial weeds on meso- to eutrophic soils (e.g. *Hordeum murinum* and *S. pratensis*).

Even if the ground vegetation of a vineyard could be associated to a semi-natural pasture or an extensively managed meadow, there are slight differences with these vegetation types, due to the particular management pressure and environmental conditions which have selected physiological, morphological and dispersal life traits of plants. Accordingly, although our plant indicator species may to some extent be associated to potential natural plant communities, the reference to single species is more pertinent, as recommended by Rosenthal (2003). Moreover, plant indicator species selected by Indicator Value analysis consists of a list of species significantly associated with each target group of sampling plots, which does not mean that the species must co-occur in the same location (de Cáseres *et al*., 2012). For these reasons, when a new sampling plot is monitored, the greater the number of indicator species recorded, the higher the confidence of its assignment to the target group for high or mid-to-high biodiversity level.

Implementation for biodiversity conservation

Payments for environmental services (PES) are a commonly used policy instrument throughout the world to help reach biodiversity conservation goals in agroecosystems (Ferraro and Kiss, 2002; Jack *et al*., 2008), despite the definition of PES has been for the most part implicit (Sommerville *et al*., 2009). An effective list of indicator species of distinct facets of biodiversity may represent a key tool to assess the status and trends of biodiversity and to quantify the ecological quality of a field (Wittig *et al*., 2006). Unfortunately, taxonomic diversity and vulnerability of species to extinction (Red Lists) are the only measures routinely taken into account in many biodiversity monitoring programs (Vandewalle *et al*., 2010). However, vulnerable species are often too rare to be considered the only important plant species when determining ecological quality (Rosenthal, 2003). In fact, in a survey carried out in Austrian meadows, Zechmeister *et al*. (2003) concluded that Red List species are not appropriate in evaluating intensively used agricultural meadows; moreover the authors observed no correlation between the amount of subsidies and plant species richness in the investigated meadows. In Switzerland, the biomonitoring of ecological quality of vineyards to grant subsidies to landowners is currently based on species of conservation concern, such as Red List species and species at high risk of extinction. Some difficulties might arise, though, when applying this type of biomonitoring protocol, because it mainly focuses on conservation-relevant aspects without completely reflect the importance of ecosystem services provided by the entire plant community.

The indicator species selected in our study are, instead, rather abundant and representative of each habitat type within vineyards, and they provide a complementary list of species related with two important biodiversity facets. As stressed by Vandewalle *et al*. (2010), in biodiversity monitoring schemes an integrated approach including different facets of biodiversity should be considered, while within biodiversity conservation strategies more than one objective should be covered. Our results emphasize the need to consider functional aspects of biodiversity in diversity-conservation strategies when the objectives are to preserve both diversity of taxa and ecosystem functioning (Cadotte *et al*., 2011). Finally, we suggest reconsidering the current official species list for the biomonitoring of ecological quality in vineyards in order to grant effective subsidies to landowners. In order to promote three important aspects of biodiversity, we propose to set up a list of indicators composed of two groups of species: (1) vulnerable species, as far as possible, from their own eco-geographic area using the national or sub-national Red List; and (2) the most abundant plant species associated with a high level of both taxonomic and functional biodiversity.

We believe that landowners and farmers should be motivated to maintain a traditional farming style through more focused (and possibly more understandable) subsidy policies, which encourage more eco-sustainable approaches. The willingness to implement our findings in the current monitoring protocol will inevitably have political and economical implications when evaluating possible trade-offs between conservation aspects and ecosystem functioning issues (Lavorel and Grigulis, 2012).

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Appendices

Figure 4:A1 Location of the 48 vineyards along the main vine growing area (light grey area) in Southern Switzerland.

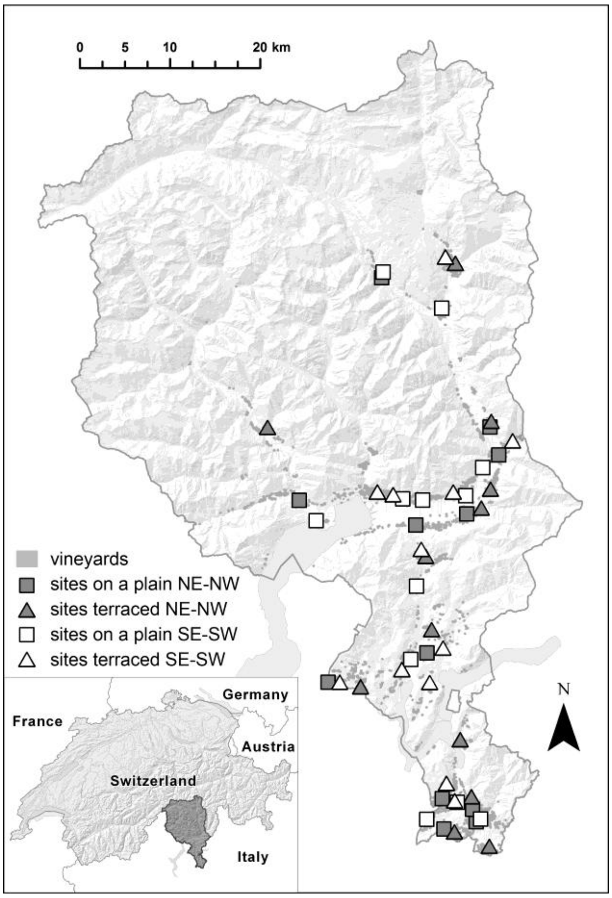


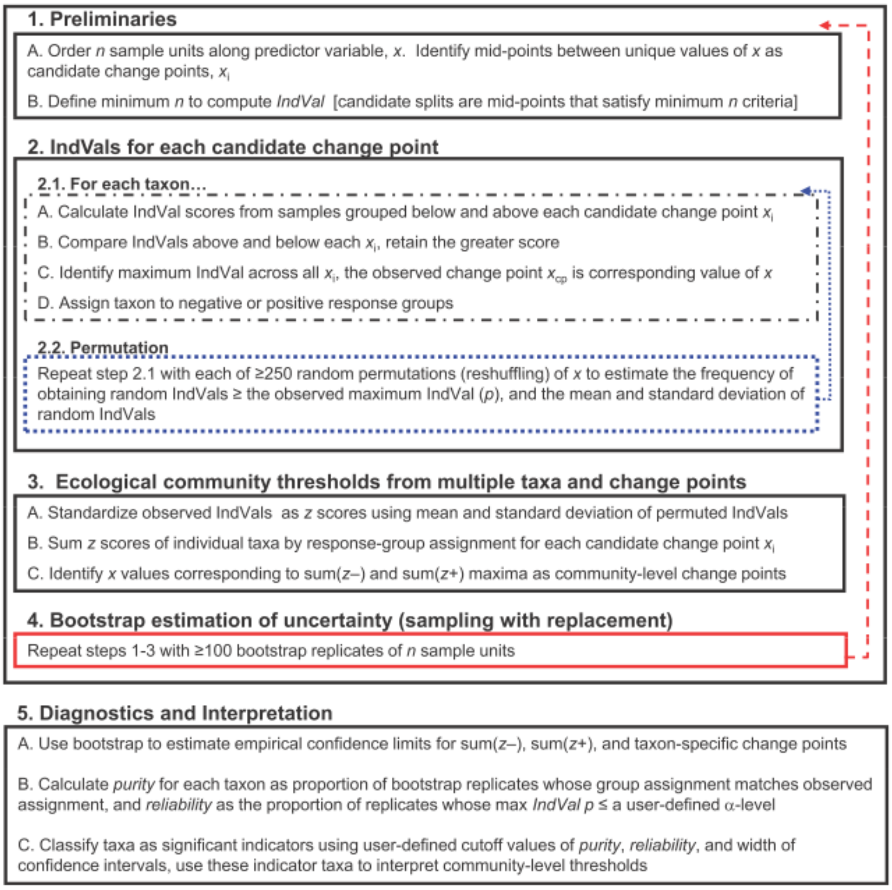
Table 4:A1 Hierarchical sampling design based on three main variables: the Aspect, Slope and surrounding Landscape of each study site. For each combination of variables, 4 study sites were selected by ArcGis 10 (ESRI, 2011).

		Study sites			
	Aspect	SE-SW		NE-NW	
	Slope	terraced	plain	terraced	plain
Landscape	Forest	4	4	4	4
	Settlements	4	4	4	4
	Open areas	4	4	4	4
		12	12	12	12

Material 4:A2. Rationale for TITAN analysis.

TITAN is a method that effectively improves the nonparametric change-point analysis (nCPA) (Quian et al., 2003; King and Richardson, 2003), which is a technique of classification and divisive partitioning of ecological data. For example, let species abundance be an ecological response variable. If observations are ordered along an explanatory gradient, then a changepoint is a value that separates the data into the two groups that have the greatest difference in means and/or variances. This difference is measured by *deviance* (the degree of within-group variance relative to the between-group variance). The nCPA analysis examines each value along the explanatory gradient and picks up the value that maximizes the reduction in deviance. TITAN improves nCPA analysis by using IndVal scores instead of deviance reduction and, then, by replacing the aggregate, community-level, dissimilarity response of nCPA with the species-specific changepoints. TITAN splits sampling plots into 2 groups at the value of an explanatory variable that maximizes association of each species with 1 side of the partition. Association is measured by species abundances weighted by their occurrence in each partition (Dufrêne and Legendre 1997) and standardized as z-scores to facilitate cross-species comparison via permutation of sampling plots along the explanatory gradient. The significance of change in robust indicator species is assessed by *purity* and *reliability* indices and narrow quantiles of changepoint location across bootstrap replicates. Correspondence in the distribution of changepoint locations for pure and reliable taxa and narrow bootstrap quantiles around a distinct peak in normalized changes summed across species (i.e., sum[z]) are used to infer evidence for thresholds in community composition and structure. TITAN distinguishes declining and increasing species and tracks the cumulative responses of increasing and decreasing species in the community (sum+[z] and sum-[z]) (King and Baker, 2010; 2011; Baker and King, 2013).

Figure 4:A2 - Flow chart of Threshold Indicator Taxa ANalysis (TITAN). Fig.1 in Backer and King (2010).



1. Preliminaries

A. Order n sample units along predictor variable, x. Identify mid-points between unique values of x as candidate change points, x_i

B. Define minimum n to compute *IndVal* [candidate splits are mid-points that satisfy minimum n criteria]

2. IndVals for each candidate change point

2.1. For each taxon…

A. Calculate IndVal scores from samples grouped below and above each candidate change point x_i

B. Compare IndVals above and below each x_i, retain the greater score

C. Identify maximum IndVal across all x_i, the observed change point x_{cp} is corresponding value of x

D. Assign taxon to negative or positive response groups

2.2. Permutation

Repeat step 2.1 with each of ≥250 random permutations (reshuffling) of x to estimate the frequency of obtaining random IndVals ≥ the observed maximum IndVal (p), and the mean and standard deviation of random IndVals

3. Ecological community thresholds from multiple taxa and change points

A. Standardize observed IndVals as z scores using mean and standard deviation of permuted IndVals

B. Sum z scores of individual taxa by response-group assignment for each candidate change point x_i

C. Identify x values corresponding to sum($z-$) and sum($z+$) maxima as community-level change points

4. Bootstrap estimation of uncertainty (sampling with replacement)

Repeat steps 1-3 with ≥100 bootstrap replicates of n sample units

5. Diagnostics and Interpretation

A. Use bootstrap to estimate empirical confidence limits for sum($z-$), sum($z+$), and taxon-specific change points

B. Calculate *purity* for each taxon as proportion of bootstrap replicates whose group assignment matches observed assignment, and *reliability* as the proportion of replicates whose max *IndVal* $p \leq$ a user-defined α-level

C. Classify taxa as significant indicators using user-defined cutoff values of *purity*, *reliability*, and width of confidence intervals, use these indicator taxa to interpret community-level thresholds

Finally, TITAN detects ecological community thresholds, i.e. a transition point of relatively rapid change in both occurrence frequency and relative abundance of plant species along a given environmental gradient.

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Table 4:A3 List of identified taxa in 120 sampling plots in vineyards of southern Switzerland surveyed in 2011.

Species
Achillea millefolium aggr.
Aegopodium podagraria L.
Agrostis capillaris L.
Agrimonia eupatoria L.
Agrostis gigantea Roth
Agrostis stolonifera L.
Ailanthus altissima (Mill.) Swingle
Ajuga reptans L.
Allium oleraceum L.
Allium vineale L.
Alopecurus geniculatus L.
Amaranthus blitum L.
Amaranthus bouchonii Thell.
Amaranthus retroflexus L.
Anagallis arvensis L.
Anemone nemorosa L.
Anthoxanthum odoratum L.
Anthyllis vulneraria L. s.str.
Aphanes australis Rydb.
Arabidopsis thaliana (L.) Heynh.
Arenaria leptoclados (Rchb.) Guss.
Arenaria serpyllifolia L.
Arrhenatherum elatius (L.) J. & C. Presl
Artemisia verlotiorum Lamotte
Asplenium adiantum-nigrum L.
Asparagus officinalis L.
Asplenium trichomanes L.
Astragalus glycyphyllos L.
Athyrium filix-femina (L.) Roth
Bellis perennis L.
Bidens bipinnata L.
Bidens frondosa L.
Bothriochloa ischaemum (L.) Keng
Brachypodium pinnatum (L.) P. Beauv.
Bromus catharticus Vahl
Bromus erectus Huds. s.l.
Bromus hordeaceus L.
Bromus sterilis L.
Calystegia sepium (L.) R. Br.
Calystegia silvatica (Kit.) Griseb.
Campanula patula L. s.l.
Campanula rapunculus L.
Capsella bursa-pastoris (L.) Medik.
Capsella rubella Reut.
Carex caryophyllea Latourr.
Cardamine hirsuta L.
Carex hirta L.
Carex muricata aggr.
Cedrus deodara (Roxb.) G.Don
Centaurea nigrescens Willd.
Centaurea scabiosa L. s.str.
Centaurea triumfettii All.
Cerastium fontanum Baumg. s.l.
Chenopodium album L.
Chelidonium majus L.
Chenopodium polyspermum L.
Chrysopogon gryllus (L.) Trin.
Cirsium arvense (L.) Scop.
Clematis vitalba L.
Clinopodium vulgare L.
Commelina communis L.
Convolvulus arvensis L.
Conyza canadensis (L.) Cronquist
Cornus sanguinea L.
Crepis biennis L.
Crepis capillaris Wallr.
Cruciata glabra (L.) Ehrend.
Cruciata laevipes Opiz
Cynodon dactylon (L.) Pers.
Cyperus esculentus L.
Cytisus scoparius (L.) Link
Dactylis glomerata L.
Daucus carota L.
Dianthus carthusianorum subsp. vaginatus (Chaix) S
Digitaria sanguinalis (L.) Scop.
Duchesnea indica (Andrews) Focke
Echinochloa crus-galli (L.) P. Beauv.
Echium vulgare L.
Elymus repens (L.) Gould
Epilobium collinum C. C. Gmel.
Epilobium parviflorum Schreb.

Species
Epilobium tetragonum L. s.str.
Equisetum arvense L.
Erigeron annuus (L.) Desf. s.l.
Euonymus europaeus L.
Eupatorium cannabinum L.
Euphorbia cyparissias L.
Euphorbia helioscopia L.
Euphorbia maculata L.
Euphorbia peplus L.
Festuca arundinacea Schreb. s.l.
Festuca ovina aggr.
Festuca pratensis Huds. s.l.
Festuca rubra aggr.
Fragaria x ananassa Duchesne
Fragaria vesca L.
Fraxinus excelsior L.
Galinsoga ciliata (Raf.) S. F. Blake
Galinsoga parviflora Cav.
Galium aparine L.
Galium mollugo aggr.
Galium verum subsp. wirtgenii (F. W. Schultz) Obor
Geranium columbinum L.
Geranium dissectum L.
Geranium molle L.
Geranium pyrenaicum Burm. f.
Geranium robertianum L. s.str.
Geranium rotundifolium L.
Geranium sanguineum L.
Geum urbanum L.
Glechoma hederacea L. s.l.
Hedera helix L.
Helianthemum nummularium (L.) Mill. s.str.
Heracleum sphondylium L. s.l.
Hibiscus syriacus L.
Hieracium pilosella L.
Hippocrepis comosa L.
Holcus lanatus L.
Holcus mollis L.
Hordeum murinum L. s.l.
Humulus lupulus L.
Hypericum perforatum L. s.str.
Hypochaeris radicata L.
Ilex aquifolium L.
Jasione montana L.
Juglans regia L.
Juncus tenuis Willd.
Knautia arvensis (L.) Coult.
Knautia dipsacifolia Kreutzer s.l.
Lactuca serriola L.
Lamium album L.
Lamium amplexicaule L.
Lamium galeobdolon subsp. flavidum (F. Herm.) Á. & D. Löve
Lamium purpureum L.
Lapsana communis L. s.l.
Lathyrus latifolius L.
Lathyrus pratensis L.
Leontodon autumnalis L.
Leontodon hispidus L. s.l.
Lepidium virginicum L.
Leucanthemum vulgare Lam.
Linaria vulgaris Mill.
Lolium multiflorum Lam.
Lolium perenne L.
Lonicera japonica Thunb.
Lotus corniculatus aggr.
Luzula campestris (L.) DC.
Luzula multiflora (Ehrh.) Lej.
Lysimachia nummularia L.
Lythrum salicaria L.
Malva neglecta Wallr.
Medicago lupulina L.
Medicago sativa L.
Mercurialis annua L.
Molinia arundinacea Schrank
Muhlenbergia schreberi J. F. Gmel.
Muscari comosum (L.) Mill.
Myosotis arvensis Hill
Myosotis ramosissima Rochel
Myosoton aquaticum (L.) Moench
Oenothera biennis aggr.
Onobrychis viciifolia Scop.
Ornithogalum umbellatum L.
Ostrya carpinifolia Scop.
Oxalis corniculata L.
Oxalis stricta L.
Oxalis violacea L.
Panicum capillare L.
Papaver dubium L. s.str.
Parietaria judaica L.
Parietaria officinalis L.
Parthenocissus inserta (A. Kern.) Fritsch
Parthenocissus tricuspidata (Siebold & Zucc.) Plan
Peucedanum oreoselinum (L.) Moench
Phyteuma betonicifolium Vill.
Phytolacca americana L.
Picris hieracioides L. s.str.
Pimpinella saxifraga L.
Plantago lanceolata L.
Plantago major L. s.l.
Poa annua L.
Poa pratensis aggr.
Poa trivialis L. s.l.
Polygonatum multiflorum (L.) All.
Polygonum aviculare aggr.
Polygonum persicaria L.
Portulaca oleracea L. s.l.
Potentilla argentea L.
Potentilla erecta (L.) Raeusch.
Potentilla reptans L.
Primula acaulis (L.) L.
Prunella vulgaris L.
Prunus avium L.
Pteridium aquilinum (L.) Kuhn
Quercus robur L.
Ranunculus acris L. s.l.
Ranunculus bulbosus L.
Ranunculus repens L.
Ranunculus sardous Crantz
Robinia pseudoacacia L.
Rorippa palustris (L.) Besser
Rorippa sylvestris (L.) Besser
Rubus fruticosus aggr. sensu Landolt
Rumex acetosa L.
Rumex acetosella L. s.str.
Rumex obtusifolius L.
Rumex scutatus L.
Sagina apetala Ard. s.l.
Salvia pratensis L.
Sanguisorba minor Scop. s.l.
Scabiosa columbaria L. s.l.
Scrophularia nodosa L.
Securigera varia (L.) Lassen
Sedum cepaea L.
Sedum dasyphyllum L.
Sedum telephium subsp. maximum (L.) Kirschl.
Senecio vulgaris L.
Setaria pumila (Poir.) Roem. & Schult.
Setaria viridis (L.) P. Beauv.
Silene dioica (L.) Clairv.

Species

Silene nutans L. s.l.
Silene pratensis (Rafn) Godr.
Silene rupestris L.
Silene vulgaris (Moench) Garcke s.l.
Solanum chenopodioides Lam.
Solanum nigrum L.
Sonchus asper Hill
Sonchus oleraceus L.
Stachys officinalis (L.) Trevis. s.l.
Stachys recta L. s.str.
Stellaria graminea L.
Stellaria media aggr.
Taraxacum officinale aggr.

Species

Teucrium chamaedrys L.
Thalictrum minus L. s.l.
Thymus pulegioides L.
Tradescantia virginiana L.
Trifolium campestre Schreb.
Trifolium pratense L. s.str.
Trifolium repens L. s.str.
Trisetum flavescens (L.) P. Beauv.
Urtica dioica L.
Valerianella locusta (L.) Laterr.
Verbascum lychnitis L.
Veronica arvensis L.
Veronica chamaedrys L.

Species

Veronica officinalis L.
Veronica persica Poir.
Veronica serpyllifolia L. s.l.
Vicia sativa subsp. nigra (L.) Ehrh.
Vicia cracca L. s.str.
Vicia hirsuta (L.) Gray
Vicia sepium L.
Vinca minor L.
Vincetoxicum hirundinaria Medik.
Viola arvensis Murray
Viola sp.
Vitis vinifera L.
Vulpia myuros (L.) C. C. Gmel.

Material 4:A4 Results of TITAN analysis.

For functional richness (FRic), the TITAN analysis revealed numerous increasing plant species (64) with changepoints distributed from 0.008 to 0.21 and a sum(z+) changepoint of 0.07. Asynchrony among increasing taxon changepoints and broad confidence limits revealed a relatively weak aggregate signal of community change. An opposite trend was observed when considering other biodiversity measures, e.g. for functional divergence the positive (z+) indicator taxa increased sharply between 0.77 and 0.91, resulting in a sum(z+) changepoint of 0.86. The strong synchrony of change in positive indicator taxa of the last five measures was consistent with a community threshold based on this index.

Table 4:AD.1 - Community threasold results from each biodiversity index calculated by Threshold Indicator Taxa ANalysis (TITAN) (**cp**= observed community thresholds, sum- and sum+, and their bootstrap 5th, 10th, 50th,90th and 95th quantiles).

Trait	cp		0.05	0.1	0.5	0.9	0.95
FRic	**sumz-**	**0.031**	**0.002461**	**0.002998**	**0.021142**	**0.038714**	**0.042452**
FRic	**sumz+**	**0.074**	**0.043037**	**0.043037**	**0.043037**	**0.14723**	**0.159591**
FDiv	**sumz-**	**0.823**	**0.788485**	**0.799583**	**0.823081**	**0.852875**	**0.852875**
FDiv	**sumz+**	**0.862**	**0.852875**	**0.852875**	**0.862225**	**0.882436**	**0.890549**
Rao	**sumz-**	**0.029**	**0.024237**	**0.025014**	**0.028853**	**0.031858**	**0.031858**
Rao	**sumz+**	**0.036**	**0.031858**	**0.031858**	**0.03589**	**0.044174**	**0.044786**
Ric	**sumz-**	**28.000**	**18.475**	**20**	**28**	**30**	**30**
Ric	**sumz+**	**33.000**	**30.5**	**30.5**	**30.5**	**38.5**	**41**
Sim	**sumz-**	**0.940**	**0.9027**	**0.909173**	**0.935192**	**0.940893**	**0.9415**
Sim	**sumz+**	**0.959**	**0.942498**	**0.942498**	**0.942498**	**0.954966**	**0.958508**
Sha	**sumz-**	**3.045**	**2.516585**	**2.599696**	**2.920312**	**3.038819**	**3.044986**
Sha	**sumz+**	**3.166**	**3.050334**	**3.050334**	**3.050334**	**3.240165**	**3.280133**

Table 4:A4 - Threshold Indicator Taxa ANalysis (TITAN) results of significant z- and z+ for each plant species and biodiversity indices considered.

+/- = z- decreasing species; z+ increasing species. **freq**= number of non-zero abundance values per species. **IndVal**= Dufrêne & Legendre (1997) IndVal statistic, scaled 0-100%. **pval**= (number of random IndVals >= observed IndVal)/numprm. **z**= IndVal z score. **cp**= changepoint value for each taxon. **5%, 95%**= bootstrap confidence interval (median among 500 simulation iterations) of changepoint. **purity**= is the mean proportion of correct response direction (z+ or z-) assignments. **Reliability**= is the mean proportion of p-values ≤ 0.05 or ≤ 0.01 among 500 simulation iterations.

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
FRic	Bromus.catharticus	z-	6	12.11	0.004	4.26	0.028885	0.00109	0.043556	0.998	0.922	0.698
FRic	Lamium.purpureum	z-	44	37.47	0.008	4.06	0.043037	0.008201	0.086648	0.996	0.958	0.824
FRic	Achillea.millefolium	z+	55	50.39	0.004	6.74	0.091823	0.036835	0.121929	1	1	0.996
FRic	Aegopodium.podagraria	z+	31	30.08	0.004	4.67	0.022184	0.017204	0.249401	1	0.986	0.848
FRic	Anthoxanthum.odoratum	z+	45	42.13	0.004	5	0.014972	0.012964	0.121929	1	1	0.958
FRic	Arrhenatherum.elatius	z+	56	57.16	0.004	9.14	0.034152	0.023408	0.105883	1	1	1
FRic	Artemisia.verlotiorum	z+	44	47.2	0.008	4.31	0.155068	0.007371	0.225359	1	1	0.882
FRic	Astragalus.glycyphyllos	z+	5	25.47	0.008	8.73	0.193315	0.096045	0.235174	0.99	0.964	0.882
FRic	Brachypodium.pinnatum	z+	24	39.12	0.004	8.18	0.091823	0.038663	0.118505	1	1	0.992
FRic	Bromus.sterilis	z+	27	41.47	0.004	8.23	0.068026	0.050856	0.146619	1	1	0.998
FRic	Calystegia.sepium	z+	14	34.14	0.004	4.94	0.205014	0.044126	0.274822	0.986	0.948	0.724
FRic	Calystegia.silvatica	z+	5	30.18	0.004	9.68	0.171217	0.088312	0.249401	0.998	0.964	0.888
FRic	Campanula.patula	z+	6	38.28	0.004	11.88	0.171217	0.134601	0.249401	0.996	0.988	0.958
FRic	Carex.hirta	z+	27	26.42	0.016	3.28	0.022184	0.014972	0.171217	0.988	0.96	0.612
FRic	Carex.muricata	z+	14	15.22	0.012	3.37	0.043521	0.022757	0.274822	0.99	0.944	0.718
FRic	Centaurea.nigrescens	z+	25	32.49	0.004	7.15	0.053997	0.037647	0.225359	1	1	0.984
FRic	Clinopodium.vulgare	z+	29	35.45	0.004	7.04	0.076605	0.036835	0.155068	1	1	0.986
FRic	Convolvulus.arvensis	z+	64	48.49	0.012	3.3	0.008201	0.002979	0.112435	0.986	0.96	0.724
FRic	Crepis.capillaris	z+	58	74.83	0.004	8.6	0.152732	0.022788	0.179342	1	1	1
FRic	Cruciata.glabra	z+	16	33.65	0.004	8.88	0.091823	0.042452	0.235174	1	0.998	0.99
FRic	Dactylis.glomerata	z+	28	31.98	0.004	6.68	0.035666	0.030306	0.087636	0.998	0.998	0.974
FRic	Daucus.carota	z+	20	61.86	0.004	9.98	0.205014	0.052923	0.274822	1	1	1
FRic	Dianthus.carthusianorum	z+	3	12.5	0.004	6.98	0.114416	0.043037	0.225359	0.964	0.938	0.796
FRic	Echium.vulgare	z+	19	25.58	0.016	3.39	0.118505	0.019446	0.155068	0.99	0.968	0.744
FRic	Erigeron.annuus	z+	70	59.28	0.004	7.78	0.043037	0.031301	0.164676	1	1	1
FRic	Festuca.ovina	z+	28	37.63	0.004	4.65	0.121929	0.04548	0.235174	0.966	0.956	0.854
FRic	Festuca.pratensis	z+	11	14.97	0.004	3.57	0.074048	0.038714	0.215153	0.99	0.938	0.786
FRic	Fragaria.vesca	z+	25	47.26	0.004	5.55	0.171217	0.03019	0.249401	1	0.998	0.97
FRic	Fraxinus.excelsior	z+	7	13.51	0.012	3.46	0.105883	0.036127	0.205521	0.986	0.9	0.684
FRic	Galium.mollugo	z+	67	62.98	0.004	10.49	0.046378	0.031353	0.126287	1	1	1
FRic	Galium.verum	z+	6	11.61	0.008	3.78	0.074048	0.038663	0.134881	0.998	0.908	0.656
FRic	Geranium.columbinum	z+	5	29.48	0.004	8.97	0.205014	0.074476	0.235174	0.99	0.952	0.838
FRic	Hedera.helix	z+	24	34.6	0.004	6.66	0.078747	0.028839	0.09796	1	1	0.976
FRic	Heracleum.sphondylium	z+	9	13.24	0.016	3.83	0.036835	0.034152	0.274822	1	0.974	0.77

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
FRic	Holcus.lanatus	z+	54	41.24	0.004	4.09	0.023978	0.018084	0.225359	0.996	0.984	0.892
FRic	Hypericum.perforatum	z+	5	32.5	0.008	8.46	0.215153	0.078747	0.274822	0.992	0.934	0.834
FRic	Hypochaeris.radicata	z+	33	40.35	0.004	4.09	0.155068	0.015884	0.274822	0.976	0.954	0.756
FRic	Knautia.dipsacifolia	z+	7	23.38	0.004	5.05	0.179342	0.03769	0.225359	0.998	0.938	0.802
FRic	Lapsana.communis	z+	11	61.69	0.004	10.88	0.215153	0.087415	0.274822	1	0.998	0.958
FRic	Lathyrus.pratensis	z+	15	24.39	0.004	5.16	0.074048	0.035112	0.11462	1	1	0.982
FRic	Leucanthemum.vulgare	z+	10	23.2	0.004	5.9	0.134601	0.041271	0.274822	0.97	0.942	0.786
FRic	Lotus.corniculatus	z+	25	44.91	0.004	7.9	0.11039	0.046333	0.205014	1	1	0.994
FRic	Luzula.campestris	z+	7	11.93	0.008	3.45	0.088312	0.035112	0.152732	0.992	0.91	0.634
FRic	Malva.neglecta	z+	11	15.07	0.004	3.26	0.034152	0.027978	0.100528	0.996	0.956	0.634
FRic	Oxalis.stricta	z+	73	49.24	0.004	3.39	0.018084	0.002624	0.171624	0.984	0.96	0.762
FRic	Parthenocissus.inserta	z+	6	22.6	0.004	6.87	0.171217	0.052923	0.249401	0.996	0.956	0.836
FRic	Peucedanum.oreoselinum	z+	19	25.2	0.004	4.46	0.091823	0.03123	0.146924	0.968	0.914	0.744
FRic	Picris.hieracioides	z+	6	10.34	0.004	3.49	0.044214	0.042452	0.274822	0.992	0.91	0.694
FRic	Primula.acaulis	z+	16	50.11	0.004	7.89	0.205014	0.03769	0.274822	1	0.998	0.97
FRic	Rubus.fruticosus	z+	13	55.98	0.004	10.62	0.179342	0.076605	0.235174	1	1	1
FRic	Rumex.acetosella	z+	26	36.99	0.008	4.09	0.155068	0.019377	0.235174	0.992	0.988	0.888
FRic	Sanguisorba.minor	z+	11	23.4	0.004	7.28	0.057355	0.052923	0.225359	1	0.998	0.992
FRic	Securigera.varia	z+	5	15.3	0.004	5.25	0.11039	0.053695	0.193315	0.99	0.93	0.756
FRic	Setaria.viridis	z+	7	13.82	0.004	4.61	0.068026	0.042452	0.114416	0.998	0.968	0.796
FRic	Silene.pratensis	z+	34	40.59	0.004	6.73	0.074048	0.048552	0.235174	1	1	0.98
FRic	Silene.vulgaris	z+	45	35.52	0.004	4.28	0.043037	0.014938	0.054165	0.986	0.968	0.862
FRic	Thalictrum.minus	z+	19	23.42	0.004	5.43	0.043037	0.033522	0.249401	0.99	0.988	0.96
FRic	Thymus.pulegioides	z+	14	21.01	0.004	5.86	0.044214	0.040586	0.140194	1	0.994	0.96
FRic	Trifolium.pratense	z+	35	28.26	0.012	3.3	0.043521	0.014872	0.274822	0.984	0.94	0.77
FRic	Urtica.dioica	z+	38	34.76	0.004	4.5	0.046378	0.018084	0.250672	0.996	0.992	0.908
FRic	Veronica.chamaedrys	z+	16	28.26	0.004	6.23	0.088312	0.074048	0.225359	0.99	0.978	0.906
FRic	Vicia.angustifolia	z+	40	44.28	0.004	6.63	0.031985	0.023408	0.225359	1	1	0.998
FRic	Vicia.cracca	z+	28	29.74	0.004	4.18	0.019446	0.017204	0.249401	0.998	0.998	0.926
FRic	Vicia.hirsuta	z+	10	18.96	0.004	5.28	0.074499	0.040557	0.102131	1	0.996	0.928
FRic	Vicia.sepium	z+	15	18.29	0.016	3.2	0.023978	0.019446	0.164676	1	0.98	0.728
FRic	Vincetoxicum.hirundinaria	z+	8	18.21	0.004	4.91	0.112435	0.03769	0.274822	1	0.972	0.822
FDiv	Achillea.millefolium	z-	55	38.31	0.008	3.51	0.841475	0.798023	0.890583	0.978	0.946	0.726
FDiv	Anthoxanthum.odoratum	z-	45	39.86	0.004	5.26	0.828842	0.789845	0.867088	0.998	0.986	0.934
FDiv	Arrhenatherum.elatius	z-	56	40.88	0.004	4.41	0.858292	0.822953	0.887574	0.992	0.972	0.864
FDiv	Brachypodium.pinnatum	z-	24	27.69	0.004	5.11	0.855403	0.827567	0.872765	0.998	0.998	0.936
FDiv	Carex.caryophyllea	z-	16	23.19	0.004	4.48	0.820159	0.772674	0.861194	0.996	0.986	0.856
FDiv	Carex.hirta	z-	27	24.99	0.012	3.23	0.829485	0.772674	0.881897	0.978	0.944	0.69
FDiv	Dactylis.glomerata	z-	28	28.74	0.004	5.15	0.853895	0.787009	0.85989	0.986	0.966	0.842
FDiv	Echium.vulgare	z-	19	30.81	0.004	7.09	0.823081	0.772674	0.837332	1	0.996	0.936
FDiv	Hieracium.pilosella	z-	5	29.44	0.004	8.69	0.789845	0.772674	0.815049	0.992	0.93	0.828
FDiv	Holcus.lanatus	z-	54	48.64	0.012	3.75	0.800382	0.78002	0.890167	0.994	0.99	0.856
FDiv	Hypochaeris.radicata	z-	33	48.6	0.02	2.97	0.772674	0.772674	0.888093	0.98	0.9	0.604

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
FDiv	Lathyrus.pratensis	z-	15	17.57	0.008	3.51	0.855704	0.806354	0.873479	0.988	0.914	0.632
FDiv	Leontodon.hispidus	z-	24	45.26	0.004	7.99	0.800382	0.78002	0.856278	1	1	0.992
FDiv	Peucedanum.oreoselinum	z-	19	30.23	0.004	5.28	0.816048	0.78002	0.85397	0.992	0.98	0.866
FDiv	Picris.hieracioides	z-	6	27.25	0.016	4.86	0.772674	0.772674	0.852875	1	0.914	0.67
FDiv	Plantago.lanceolata	z-	52	50.39	0.004	6.85	0.826024	0.788485	0.860025	1	1	0.982
FDiv	Ranunculus.bulbosus	z-	27	59.61	0.004	6.4	0.788485	0.772674	0.829485	0.976	0.966	0.914
FDiv	Rumex.acetosa	z-	62	50.77	0.004	4.52	0.806745	0.790326	0.862832	0.998	0.976	0.836
FDiv	Salvia.pratensis	z-	10	28.55	0.004	9.3	0.819171	0.78002	0.826652	1	0.998	0.986
FDiv	Setaria.viridis	z-	7	17.12	0.004	6.55	0.823081	0.799505	0.832965	0.988	0.94	0.826
FDiv	Silene.vulgaris	z-	45	45.7	0.004	7.3	0.855704	0.806354	0.861194	1	1	0.992
FDiv	Thalictrum.minus	z-	19	20.3	0.004	3.79	0.834381	0.78002	0.869713	0.998	0.974	0.758
FDiv	Trifolium.pratense	z-	35	31.92	0.008	3.67	0.876628	0.793262	0.881304	0.998	0.982	0.776
FDiv	Vincetoxicum.hirundinaria	z-	8	12.7	0.012	3.82	0.854739	0.772674	0.860376	0.998	0.92	0.664
FDiv	Viola.arvensis	z-	40	36.58	0.008	4.16	0.827567	0.772674	0.876628	0.99	0.97	0.838
FDiv	Bellis.perennis	z+	29	32.92	0.004	5.75	0.843833	0.828842	0.904621	1	1	0.982
FDiv	Cardamine.hirsuta	z+	43	41.33	0.004	5.28	0.844063	0.834381	0.868337	0.99	0.976	0.902
FDiv	Cerastium.fontanum	z+	54	41.11	0.004	4.08	0.854739	0.816283	0.876399	0.996	0.976	0.818
FDiv	Elymus.repens	z+	15	16.85	0.02	2.97	0.872765	0.823081	0.897671	0.982	0.908	0.608
FDiv	Euphorbia.helioscopia	z+	15	50.67	0.004	8.95	0.897671	0.861748	0.914419	0.984	0.968	0.88
FDiv	Glechoma.hederacea	z+	36	35.87	0.008	4.16	0.815433	0.807474	0.873849	0.998	0.994	0.85
FDiv	Hordeum.murinum	z+	22	29.96	0.004	5.22	0.867022	0.855656	0.904621	0.964	0.948	0.82
FDiv	Lamium.purpureum	z+	44	52.44	0.008	3.95	0.894329	0.835994	0.910661	0.95	0.93	0.804
FDiv	Lolium.multiflorum	z+	37	34.97	0.004	4.21	0.867022	0.830706	0.899022	0.956	0.944	0.812
FDiv	Medicago.lupulina	z+	7	25.88	0.012	5.72	0.896554	0.838356	0.914419	0.996	0.928	0.748
FDiv	Oenothera.biennis	z+	13	32.54	0.004	5.24	0.89242	0.841475	0.914419	0.986	0.954	0.79
FDiv	Plantago.major	z+	32	34	0.004	4.78	0.83077	0.825464	0.888812	1	1	0.956
FDiv	Poa.annua	z+	20	49.58	0.008	5.22	0.904621	0.835868	0.914419	0.982	0.942	0.786
FDiv	Poa.trivialis	z+	62	45.39	0.004	3.9	0.83077	0.806354	0.889715	0.998	0.98	0.874
FDiv	Ranunculus.repens	z+	30	28.69	0.004	4.02	0.8533	0.820159	0.881897	0.98	0.944	0.738
FDiv	Rumex.obtusifolius	z+	21	25.76	0.004	4.32	0.868337	0.826604	0.889692	0.992	0.968	0.78
FDiv	Taraxacum.officinale	z+	110	65.09	0.004	6.37	0.825589	0.789777	0.836566	1	1	0.988
FDiv	Veronica.persica	z+	71	55.12	0.004	5.65	0.860025	0.844362	0.889692	0.996	0.996	0.982
Rao	Achillea.millefolium	z-	55	42.56	0.004	4.44	0.036329	0.024995	0.041518	0.992	0.982	0.86
Rao	Agrostis.stolonifera	z-	11	14.32	0.012	3.32	0.029563	0.02259	0.037124	0.992	0.914	0.588
Rao	Anthoxanthum.odoratum	z-	45	49.57	0.004	7.06	0.036804	0.024651	0.038598	1	1	1
Rao	Arrhenatherum.elatius	z-	56	46.88	0.004	6.15	0.030147	0.025335	0.038338	1	1	0.992
Rao	Brachypodium.pinnatum	z-	24	27.77	0.012	3.22	0.025623	0.021903	0.037989	0.994	0.968	0.78
Rao	Campanula.rapunculus	z-	12	17.91	0.004	4.52	0.032443	0.02546	0.034238	0.998	0.992	0.896
Rao	Carex.caryophyllea	z-	16	38.95	0.004	5.46	0.022382	0.021273	0.034313	1	1	0.966
Rao	Centaurea.nigrescens	z-	25	31.67	0.012	3.9	0.02546	0.021273	0.039839	0.982	0.912	0.692
Rao	Clinopodium.vulgare	z-	29	29.81	0.004	4.47	0.029563	0.024237	0.03577	0.986	0.978	0.848
Rao	Dactylis.glomerata	z-	28	30.99	0.004	4.46	0.036804	0.022537	0.037989	1	1	0.954
Rao	Echium.vulgare	z-	19	52.62	0.004	6.66	0.021433	0.021207	0.03118	0.996	0.974	0.816

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
Rao	Euphorbia.cyparissias	z-	10	16.74	0.004	3.57	0.025891	0.021207	0.033664	0.988	0.908	0.656
Rao	Galium.mollugo	z-	67	53.85	0.008	3.88	0.02546	0.025014	0.040007	0.964	0.956	0.842
Rao	Holcus.lanatus	z-	54	45.73	0.004	5.99	0.029563	0.025014	0.035897	0.998	0.996	0.96
Rao	Hypochaeris.radicata	z-	33	32.95	0.004	5.79	0.032153	0.028391	0.038338	1	1	0.99
Rao	Leontodon.hispidus	z-	24	44.3	0.004	10.59	0.027498	0.021903	0.029892	1	1	0.994
Rao	Luzula.campestris	z-	7	25.99	0.008	5.29	0.022382	0.021207	0.032574	0.99	0.928	0.73
Rao	Peucedanum.oreoselinum	z-	19	25.89	0.012	4	0.027459	0.021273	0.034662	0.98	0.936	0.746
Rao	Picris.hieracioides	z-	6	13.33	0.004	4.73	0.029765	0.021903	0.030831	1	0.952	0.798
Rao	Plantago.lanceolata	z-	52	52.29	0.004	7.92	0.031339	0.02755	0.032443	1	1	1
Rao	Potentilla.reptans	z-	69	42.38	0.012	3.09	0.031858	0.023492	0.044174	0.988	0.966	0.742
Rao	Prunella.vulgaris	z-	12	23.79	0.004	6.35	0.029241	0.027498	0.030251	1	0.994	0.942
Rao	Ranunculus.acris	z-	21	22.81	0.004	3.96	0.033085	0.024651	0.03682	0.99	0.97	0.806
Rao	Ranunculus.bulbosus	z-	27	50.03	0.004	5.17	0.022382	0.021207	0.03245	0.992	0.966	0.836
Rao	Rumex.acetosa	z-	62	58.68	0.004	7.48	0.026332	0.025318	0.036042	1	1	1
Rao	Salvia.pratensis	z-	10	28.88	0.004	9.61	0.027498	0.021433	0.029887	1	1	0.99
Rao	Setaria.viridis	z-	7	11.29	0.012	3.61	0.032044	0.025361	0.032574	0.998	0.93	0.686
Rao	Silene.vulgaris	z-	45	52.38	0.004	8.41	0.027498	0.022537	0.030147	1	1	0.994
Rao	Thalictrum.minus	z-	19	21.35	0.008	3.55	0.037666	0.027097	0.038598	0.998	0.978	0.73
Rao	Trifolium.pratense	z-	35	43.09	0.004	6.9	0.028038	0.027286	0.034319	0.998	0.996	0.98
Rao	Bromus.sterilis	z+	27	28.51	0.004	4.94	0.03118	0.030147	0.045777	0.986	0.978	0.906
Rao	Capsella.bursa.pastoris	z+	35	42.68	0.004	6.67	0.03589	0.032574	0.039112	0.998	0.994	0.928
Rao	Cardamine.hirsuta	z+	43	52.49	0.004	8.31	0.03589	0.031339	0.039841	1	1	1
Rao	Cerastium.fontanum	z+	54	44.02	0.004	4.84	0.030831	0.029846	0.042801	1	0.998	0.95
Rao	Chenopodium.album	z+	13	20.97	0.004	5.26	0.031574	0.030246	0.038752	1	1	0.964
Rao	Conyza.canadensis	z+	27	24.15	0.016	2.96	0.030147	0.028399	0.044786	0.956	0.938	0.672
Rao	Cynodon.dactylon	z+	24	43.97	0.004	5.59	0.042032	0.031729	0.045777	0.982	0.968	0.876
Rao	Digitaria.sanguinalis	z+	79	49.67	0.004	4.1	0.02964	0.026765	0.043888	0.992	0.99	0.932
Rao	Echinochloa.crus.galli	z+	7	18.19	0.004	5.51	0.039654	0.030831	0.041245	0.996	0.932	0.732
Rao	Euonymus.europaeus	z+	7	32.78	0.004	8.42	0.042787	0.038598	0.045777	1	0.99	0.942
Rao	Euphorbia.helioscopia	z+	15	36.51	0.004	7.52	0.041245	0.0309	0.044174	1	1	0.986
Rao	Geranium.molle	z+	80	53.97	0.004	4.88	0.02964	0.024651	0.034242	0.998	0.998	0.962
Rao	Hordeum.murinum	z+	22	27.09	0.004	5.35	0.033489	0.030831	0.042032	0.998	0.996	0.946
Rao	Lamium.purpureum	z+	44	52.78	0.004	6.74	0.038733	0.028615	0.042611	1	1	0.99
Rao	Lapsana.communis	z+	11	32.32	0.008	4.36	0.043888	0.031852	0.045777	0.988	0.936	0.76
Rao	Medicago.lupulina	z+	7	12.96	0.016	4.27	0.032267	0.031562	0.042787	0.998	0.946	0.782
Rao	Oenothera.biennis	z+	13	19.37	0.004	5.32	0.033978	0.031339	0.044786	1	0.998	0.934
Rao	Poa.annua	z+	20	24.75	0.004	5.43	0.032267	0.028399	0.037316	0.97	0.964	0.866
Rao	Portulaca.oleracea	z+	21	30.2	0.004	5.59	0.03589	0.030583	0.03984	0.998	0.994	0.95
Rao	Senecio.vulgaris	z+	22	31.81	0.004	6.43	0.033648	0.031025	0.041518	1	1	0.99
Rao	Stellaria.media	z+	74	60.98	0.004	7.6	0.035214	0.029043	0.037124	1	1	1
Rao	Valerianella.locusta	z+	7	49.17	0.004	7.67	0.044786	0.031729	0.045777	0.998	0.934	0.774
Rao	Veronica.persica	z+	71	67.38	0.004	9.54	0.030251	0.027777	0.037316	1	1	1
Rao	Vitis.vinifera	z+	8	14.55	0.004	4.57	0.03216	0.031562	0.041526	1	0.982	0.858

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
Ric	Lamium.purpureum	z-	44	42.28	0.004	5.02	28	24.475	33	0.998	0.998	0.916
Ric	Rorippa.sylvestris	z-	7	15.22	0.004	6.65	28.5	17.5	30	1	0.978	0.842
Ric	Veronica.persica	z-	71	58.23	0.004	5.93	28	27	31	0.996	0.996	0.964
Ric	Achillea.millefolium	z+	55	51.88	0.004	7.66	28	24	40	1	1	1
Ric	Aegopodium.podagraria	z+	31	26.49	0.016	3.32	28	22.5	49.5	0.974	0.948	0.65
Ric	Agrostis.stolonifera	z+	11	31.58	0.044	3.54	50.5	27	50.5	0.992	0.93	0.516
Ric	Anthoxanthum.odoratum	z+	45	41.78	0.004	6.2	33	25	47	1	1	0.992
Ric	Arrhenatherum.elatius	z+	56	65.38	0.004	11.63	28	28	34	1	1	1
Ric	Artemisia.verlotiorum	z+	44	50.27	0.004	3.5	47.5	23	49	0.998	0.982	0.778
Ric	Astragalus.glycyphyllos	z+	5	17.86	0.004	7.22	38.5	34.5	42.5	0.996	0.966	0.868
Ric	Brachypodium.pinnatum	z+	24	32	0.004	6.07	28	27	44.575	1	1	0.99
Ric	Bromus.sterilis	z+	27	30.2	0.008	3.49	40	23	41.5	0.982	0.95	0.71
Ric	Calystegia.silvatica	z+	5	27.98	0.004	11.18	46.5	41	50.5	0.974	0.95	0.884
Ric	Campanula.patula	z+	6	27.27	0.004	10.53	41	38	49.025	0.998	0.996	0.964
Ric	Carex.caryophyllea	z+	16	20.77	0.008	4.6	33	27	41.05	1	0.998	0.85
Ric	Carex.hirta	z+	27	28.83	0.004	5.09	28.5	26	32.5	0.998	0.996	0.91
Ric	Carex.muricata	z+	14	17.19	0.004	3.99	28	27	49	0.998	0.938	0.622
Ric	Centaurea.nigrescens	z+	25	30.23	0.004	5.41	36	28	49	1	0.998	0.96
Ric	Clinopodium.vulgare	z+	29	36.53	0.004	7.61	33.5	28	46.525	1	1	0.998
Ric	Convolvulus.arvensis	z+	64	43.34	0.012	3.14	34.5	18	46.025	0.96	0.912	0.654
Ric	Crepis.capillaris	z+	58	53.67	0.004	8.06	33	27	47.5	1	1	1
Ric	Cruciata.glabra	z+	16	43.98	0.004	8.84	42	33	47	1	1	0.992
Ric	Dactylis.glomerata	z+	28	33.12	0.004	6.14	28	27.5	40.05	1	1	0.978
Ric	Daucus.carota	z+	20	55.52	0.004	8.6	47	33	50.5	1	1	0.994
Ric	Echium.vulgare	z+	19	22.86	0.004	5.03	30	28	47	0.998	0.994	0.912
Ric	Erigeron.annuus	z+	70	69.1	0.004	9.4	38.5	27	40.5	1	1	1
Ric	Festuca.ovina	z+	28	46.65	0.004	5.68	46.5	33	49.5	0.98	0.956	0.796
Ric	Fragaria.vesca	z+	25	32.06	0.004	6.23	30	28	37.5	1	1	0.982
Ric	Galium.mollugo	z+	67	59.63	0.004	9.18	31	27	36	1	1	1
Ric	Geranium.columbinum	z+	5	36.79	0.004	9.92	49	40	49.5	0.996	0.982	0.93
Ric	Holcus.lanatus	z+	54	54.25	0.004	8.47	28.5	28	34.55	1	1	1
Ric	Hypochaeris.radicata	z+	33	34.76	0.004	5.28	27	25.475	50.5	1	1	0.984
Ric	Lapsana.communis	z+	11	21.38	0.004	5.83	36	30	50.5	1	1	0.978
Ric	Lathyrus.pratensis	z+	15	29.11	0.008	5.05	41.5	28	47	0.998	0.988	0.886
Ric	Leontodon.hispidus	z+	24	23.36	0.008	3.93	31	23.5	47.5	0.988	0.936	0.666
Ric	Leucanthemum.vulgare	z+	10	43.92	0.004	7.42	49	30	50.5	1	0.988	0.904
Ric	Lotus.corniculatus	z+	25	46.14	0.004	8.89	37.5	31	42.6	1	1	1
Ric	Luzula.campestris	z+	7	31.81	0.004	8.33	47	31	49	0.986	0.954	0.8
Ric	Oxalis.stricta	z+	73	52.41	0.004	4.97	28	25	47	1	0.994	0.908
Ric	Peucedanum.oreoselinum	z+	19	19.25	0.028	2.74	39.5	24	47.55	0.994	0.904	0.61
Ric	Picris.hieracioides	z+	6	27.5	0.008	7.28	47.5	33	49	1	0.978	0.834
Ric	Plantago.lanceolata	z+	52	36.02	0.02	2.95	27	23	49.5	0.99	0.95	0.68
Ric	Potentilla.erecta	z+	5	12.82	0.004	5.99	34	33	50.5	0.99	0.912	0.734

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
Ric	Potentilla.reptans	z+	69	53.55	0.004	3.52	44.5	18	49.5	0.954	0.952	0.832
Ric	Primula.acaulis	z+	16	36.97	0.004	8.52	36	34	50.5	1	1	0.998
Ric	Ranunculus.bulbosus	z+	27	25.98	0.004	3.51	32.5	23.5	50.5	0.99	0.978	0.77
Ric	Rubus.fruticosus	z+	13	25.34	0.004	7.07	34	30	47	1	1	0.994
Ric	Rumex.acetosa	z+	62	47.64	0.004	5.62	28.5	20.95	33.05	1	0.998	0.992
Ric	Rumex.acetosella	z+	26	36.02	0.016	3.2	47.5	24.975	49	0.986	0.958	0.706
Ric	Salvia.pratensis	z+	10	16.03	0.004	4.58	31	30	47	0.99	0.96	0.818
Ric	Sanguisorba.minor	z+	11	23.89	0.004	5.75	39.5	30	47.55	1	0.994	0.936
Ric	Securigera.varia	z+	5	15.2	0.008	5.24	42	31.5	47	0.996	0.9	0.706
Ric	Setaria.viridis	z+	7	14.89	0.004	5.22	33	31	40	1	0.984	0.846
Ric	Silene.pratensis	z+	34	38.14	0.004	5.18	38.5	27	46.025	1	0.998	0.962
Ric	Silene.vulgaris	z+	45	43.41	0.004	6.49	28	27	40.525	1	1	0.986
Ric	Thalictrum.minus	z+	19	37.65	0.004	4.61	47.5	28	50.5	1	0.986	0.884
Ric	Thymus.pulegioides	z+	14	23.04	0.004	6.46	33	30	47	1	0.998	0.96
Ric	Trifolium.pratense	z+	35	37.85	0.004	5.13	39.5	25	49.5	1	0.994	0.898
Ric	Urtica.dioica	z+	38	38.02	0.004	5.64	30.5	27	34.075	1	0.998	0.964
Ric	Veronica.chamaedrys	z+	16	30.51	0.012	3.66	47.5	28	49.5	0.99	0.95	0.75
Ric	Vicia.angustifolia	z+	40	43.08	0.004	4.36	42	23.5	49	0.996	0.986	0.888
Ric	Vicia.cracca	z+	28	31.5	0.004	5.2	28	26	49.025	1	1	0.97
Ric	Vicia.sepium	z+	15	19.74	0.004	4.27	28	28	40.525	1	0.998	0.874
Ric	Vincetoxicum.hirundinaria	z+	8	18.18	0.004	6.38	34	31	46.525	0.998	0.986	0.936
Ric	Viola.sp.	z+	25	52.27	0.016	3.83	50.5	27	50.5	0.998	0.982	0.798
Sim	Bromus.catharticus	z-	6	12	0.012	4.21	0.93691	0.930915	0.942129	1	0.924	0.716
Sim	Chenopodium.album	z-	13	25.78	0.012	4.56	0.911525	0.892154	0.949813	0.968	0.9	0.686
Sim	Lamium.purpureum	z-	44	40.71	0.004	5.73	0.936283	0.930701	0.957433	0.998	0.998	0.924
Sim	Rorippa.sylvestris	z-	7	14	0.004	5.19	0.93691	0.929989	0.940893	1	0.976	0.858
Sim	Veronica.persica	z-	71	56.44	0.004	6.42	0.940893	0.926501	0.948379	1	1	0.994
Sim	Achillea.millefolium	z+	55	50.26	0.004	7.47	0.940893	0.930701	0.960162	1	1	1
Sim	Aegopodium.podagraria	z+	31	31.96	0.004	3.55	0.919574	0.911525	0.951918	0.998	0.998	0.748
Sim	Agrostis.stolonifera	z+	11	15.01	0.012	3.48	0.939924	0.936249	0.966659	1	0.952	0.716
Sim	Anthoxanthum.odoratum	z+	45	63.58	0.004	7.88	0.960886	0.938413	0.961888	1	1	0.998
Sim	Arrhenatherum.elatius	z+	56	69.12	0.004	13.09	0.950026	0.936448	0.954966	1	1	1
Sim	Artemisia.verlotiorum	z+	44	41.04	0.008	3.54	0.919574	0.909173	0.962428	0.992	0.98	0.776
Sim	Astragalus.glycyphyllos	z+	5	14.29	0.004	6.05	0.951575	0.949802	0.960803	0.992	0.938	0.794
Sim	Brachypodium.pinnatum	z+	24	29.76	0.004	6.55	0.940893	0.935192	0.961549	1	1	0.998
Sim	Bromus.sterilis	z+	27	25.51	0.012	3.9	0.944736	0.928986	0.963952	0.994	0.97	0.754
Sim	Calystegia.silvatica	z+	5	18.06	0.004	5.83	0.960803	0.945265	0.966659	0.98	0.916	0.752
Sim	Campanula.patula	z+	6	27.59	0.004	9.33	0.960508	0.952722	0.962955	0.996	0.994	0.952
Sim	Carex.caryophyllea	z+	16	19.54	0.004	4.81	0.94658	0.934207	0.962955	1	1	0.948
Sim	Carex.hirta	z+	27	29.58	0.004	5.58	0.938413	0.934219	0.952201	1	1	0.958
Sim	Centaurea.nigrescens	z+	25	40.58	0.004	6.17	0.958508	0.936249	0.963005	1	1	0.986
Sim	Clinopodium.vulgare	z+	29	36.33	0.004	7.16	0.939924	0.933962	0.960803	1	1	1
Sim	Convolvulus.arvensis	z+	64	46.46	0.024	2.59	0.904424	0.897625	0.957433	0.984	0.932	0.602

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
Sim	Crepis.capillaris	z+	58	55.95	0.004	7.72	0.936448	0.933778	0.954298	1	1	1
Sim	Cruciata.glabra	z+	16	46.75	0.004	12.26	0.958508	0.954281	0.96566	1	1	0.998
Sim	Dactylis.glomerata	z+	28	32.11	0.004	6.18	0.942129	0.933962	0.957433	1	1	0.99
Sim	Daucus.carota	z+	20	45.31	0.004	9.23	0.960152	0.944736	0.96566	1	1	1
Sim	Dianthus.carthusianorum	z+	3	15	0.004	7.72	0.960152	0.942498	0.963952	0.968	0.936	0.758
Sim	Echium.vulgare	z+	19	34.16	0.004	4.92	0.960934	0.933571	0.962955	1	0.988	0.894
Sim	Elymus.repens	z+	15	17.86	0.008	3.41	0.930915	0.928986	0.955011	0.994	0.958	0.652
Sim	Erigeron.annuus	z+	70	69.11	0.004	7.18	0.913988	0.907249	0.954966	1	1	1
Sim	Euphorbia.cyparissias	z+	10	15.7	0.004	3.89	0.949802	0.938413	0.962955	0.984	0.954	0.76
Sim	Festuca.ovina	z+	28	30.49	0.004	3.65	0.954966	0.926024	0.962955	0.962	0.908	0.67
Sim	Fragaria.vesca	z+	25	30.34	0.004	5.4	0.936249	0.93607	0.9537	1	1	0.992
Sim	Galium.mollugo	z+	67	62.49	0.004	9.62	0.942129	0.935192	0.954612	1	1	1
Sim	Geranium.columbinum	z+	5	19.53	0.004	7.32	0.960152	0.949396	0.966659	0.998	0.948	0.82
Sim	Hieracium.pilosella	z+	5	19.5	0.004	5.43	0.960934	0.946057	0.961888	0.99	0.926	0.742
Sim	Holcus.lanatus	z+	54	53.11	0.004	8.43	0.946057	0.933789	0.952446	1	1	1
Sim	Hypochaeris.radicata	z+	33	32.14	0.004	5.07	0.936448	0.930915	0.963952	1	1	0.976
Sim	Lapsana.communis	z+	11	22.12	0.004	6.77	0.951575	0.93739	0.966659	1	0.994	0.924
Sim	Lathyrus.pratensis	z+	15	30.88	0.008	5.08	0.960508	0.940219	0.96566	1	1	0.932
Sim	Leontodon.hispidus	z+	24	44.48	0.004	5.59	0.960934	0.930859	0.962955	1	1	0.954
Sim	Leucanthemum.vulgare	z+	10	29.61	0.004	4.72	0.963952	0.936639	0.966659	1	0.984	0.854
Sim	Lotus.corniculatus	z+	25	38.31	0.004	8.09	0.94658	0.940663	0.959336	1	1	1
Sim	Oxalis.stricta	z+	73	49.63	0.004	3.89	0.936639	0.912946	0.963952	0.996	0.988	0.89
Sim	Peucedanum.oreoselinum	z+	19	32.96	0.004	5.57	0.959336	0.929279	0.96305	1	0.986	0.86
Sim	Picris.hieracioides	z+	6	23.64	0.004	9.01	0.959336	0.95028	0.96566	0.994	0.984	0.914
Sim	Plantago.lanceolata	z+	52	43.57	0.004	4.02	0.926361	0.916591	0.9624	1	0.994	0.872
Sim	Potentilla.erecta	z+	5	37.54	0.004	5.3	0.966659	0.946554	0.966659	0.994	0.938	0.774
Sim	Potentilla.reptans	z+	69	46.6	0.016	3.02	0.954612	0.895179	0.962955	0.976	0.954	0.728
Sim	Primula.acaulis	z+	16	35.45	0.004	8.74	0.952446	0.950026	0.966659	1	1	0.986
Sim	Ranunculus.bulbosus	z+	27	40.02	0.004	4.92	0.960653	0.927454	0.961549	1	1	0.954
Sim	Rubus.fruticosus	z+	13	21.94	0.004	5.37	0.94658	0.93739	0.964858	1	1	0.984
Sim	Rumex.acetosa	z+	62	58.93	0.004	6.26	0.926501	0.916591	0.951575	1	1	0.996
Sim	Rumex.acetosella	z+	26	51.34	0.016	3.86	0.96566	0.927454	0.966659	0.96	0.912	0.664
Sim	Salvia.pratensis	z+	10	26.44	0.004	6.5	0.959336	0.947367	0.961888	1	0.996	0.952
Sim	Sanguisorba.minor	z+	11	17.46	0.004	4.81	0.940893	0.93691	0.963998	1	1	0.91
Sim	Setaria.viridis	z+	7	13.46	0.004	4.46	0.945727	0.942965	0.960365	0.996	0.954	0.834
Sim	Silene.pratensis	z+	34	41.16	0.004	6.81	0.950026	0.935192	0.960934	1	1	0.988
Sim	Silene.vulgaris	z+	45	47.86	0.004	7.55	0.940219	0.932527	0.960886	1	1	1
Sim	Thalictrum.minus	z+	19	20.95	0.004	4.16	0.940219	0.929279	0.964858	1	1	0.866
Sim	Thymus.pulegioides	z+	14	21.43	0.004	5.71	0.94658	0.93607	0.9624	1	1	0.964
Sim	Trifolium.pratense	z+	35	49.8	0.004	5.93	0.960886	0.930859	0.964858	1	1	0.966
Sim	Urtica.dioica	z+	38	41.09	0.004	6.32	0.936283	0.928986	0.946644	1	1	0.99
Sim	Veronica.chamaedrys	z+	16	20.14	0.012	3.93	0.936283	0.932586	0.954281	0.998	0.99	0.86
Sim	Vicia.angustifolia	z+	40	62.52	0.012	4.18	0.964858	0.926361	0.966659	0.992	0.982	0.84

							Changepoint				**Reliability**	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
Sim	Vicia.cracca	z+	28	32.78	0.004	5.94	0.939555	0.929954	0.966659	1	1	0.962
Sim	Vicia.sepium	z+	15	17.65	0.012	2.98	0.930865	0.929222	0.964858	0.982	0.954	0.632
Sim	Vincetoxicum.hirundinaria	z+	8	17.39	0.004	6.42	0.948363	0.945704	0.964899	1	0.996	0.952
Sim	Viola.sp.	z+	25	34.42	0.008	4.78	0.954281	0.936249	0.964858	0.998	0.99	0.912
Sha	Bromus.catharticus	z-	6	11.54	0.008	4.43	2.992608	2.508606	3.041001	1	0.914	0.716
Sha	Lamium.purpureum	z-	44	40.71	0.008	4.7	2.960331	2.859414	3.24032	1	0.998	0.928
Sha	Rorippa.sylvestris	z-	7	13.73	0.004	4.87	2.988469	2.817775	3.038819	1	0.98	0.846
Sha	Veronica.persica	z-	71	56.8	0.004	6.61	3.044986	2.801589	3.109219	1	1	0.986
Sha	Achillea.millefolium	z+	55	50.96	0.004	7.57	2.961076	2.867863	3.280133	1	1	1
Sha	Aegopodium.podagraria	z+	31	31.96	0.008	3.84	2.755897	2.64204	3.166855	0.986	0.974	0.746
Sha	Agrostis.stolonifera	z+	11	14.56	0.004	3.58	2.992608	2.919853	3.586091	0.994	0.958	0.634
Sha	Anthoxanthum.odoratum	z+	45	48.54	0.004	7.49	3.128085	2.930629	3.468838	1	1	1
Sha	Arrhenatherum.elatius	z+	56	69.41	0.004	12.79	3.122161	2.934325	3.237605	1	1	1
Sha	Artemisia.verlotiorum	z+	44	40.51	0.012	3.69	2.726426	2.618868	3.523301	0.994	0.978	0.748
Sha	Astragalus.glycyphyllos	z+	5	16.67	0.004	6.83	3.240165	3.20291	3.414296	0.988	0.95	0.844
Sha	Brachypodium.pinnatum	z+	24	37.12	0.004	8.8	3.196284	2.919853	3.525075	1	1	1
Sha	Bromus.sterilis	z+	27	26.21	0.012	3.56	3.091179	2.817775	3.468838	0.996	0.968	0.77
Sha	Calystegia.silvatica	z+	5	22.92	0.004	7.52	3.427043	3.278543	3.558782	0.982	0.926	0.824
Sha	Campanula.patula	z+	6	33.33	0.004	12.9	3.414296	3.278868	3.474097	1	0.986	0.972
Sha	Campanula.rapunculus	z+	12	32.61	0.036	3.7	3.586091	2.966429	3.586091	0.968	0.908	0.664
Sha	Carex.caryophyllea	z+	16	20.73	0.004	4.73	2.961076	2.908002	3.558782	1	1	0.942
Sha	Carex.hirta	z+	27	29.54	0.004	5.62	3.050334	2.898805	3.215075	0.996	0.994	0.954
Sha	Carex.muricata	z+	14	22.22	0.024	3.26	3.414296	2.881163	3.480734	0.992	0.954	0.646
Sha	Centaurea.nigrescens	z+	25	43.04	0.004	6.68	3.402918	2.960331	3.523301	0.998	0.998	0.994
Sha	Clinopodium.vulgare	z+	29	50.68	0.004	8.3	3.391257	2.920312	3.434386	1	1	1
Sha	Convolvulus.arvensis	z+	64	41.96	0.028	2.46	3.237471	2.517004	3.434431	0.962	0.906	0.562
Sha	Crepis.capillaris	z+	58	55.95	0.004	8.05	2.961076	2.920312	3.272697	1	1	0.998
Sha	Cruciata.glabra	z+	16	46.75	0.004	11.11	3.377683	3.272372	3.558782	1	1	0.996
Sha	Dactylis.glomerata	z+	28	31.66	0.004	6.37	2.96671	2.906881	3.258308	1	1	0.998
Sha	Daucus.carota	z+	20	57.26	0.004	10.36	3.402918	3.102441	3.558782	1	1	0.998
Sha	Echium.vulgare	z+	19	26.15	0.004	5.19	3.181305	2.867863	3.558782	1	0.996	0.888
Sha	Elymus.repens	z+	15	15.89	0.02	2.92	3.087675	2.832677	3.452048	0.984	0.932	0.56
Sha	Erigeron.annuus	z+	70	64.56	0.004	8.29	3.244739	2.669065	3.346912	1	1	1
Sha	Euphorbia.cyparissias	z+	10	14.75	0.004	3.46	3.050334	2.999982	3.525075	0.998	0.958	0.712
Sha	Festuca.ovina	z+	28	40.42	0.004	5.26	3.398321	2.945246	3.480734	0.984	0.952	0.824
Sha	Fragaria.vesca	z+	25	34.61	0.004	6.37	3.141648	2.96671	3.243272	1	1	0.994
Sha	Galium.mollugo	z+	67	64.45	0.004	10.05	2.992608	2.957846	3.243272	1	1	1
Sha	Geranium.columbinum	z+	5	29.28	0.004	7.78	3.48061	3.199759	3.586091	0.992	0.94	0.852
Sha	Hieracium.pilosella	z+	5	11.9	0.008	4.77	3.173921	3.109219	3.434431	0.994	0.91	0.73
Sha	Holcus.lanatus	z+	54	57.39	0.004	8.91	3.136162	2.898805	3.186621	1	1	1
Sha	Hypochaeris.radicata	z+	33	34.57	0.004	5.45	2.920312	2.862786	3.558782	1	1	0.986
Sha	Lapsana.communis	z+	11	23.72	0.004	6.7	3.227328	3.031588	3.586091	1	1	0.948
Sha	Lathyrus.pratensis	z+	15	23.54	0.004	4.66	3.199759	2.999982	3.523301	0.996	0.982	0.888

							Changepoint				Reliability	
	Species	**+/-**	**freq**	**Ind Val**	**pval**	**z**	**cp**	**5%**	**95%**	**purity**	**≤0.05**	**≤0.01**
Sha	Leontodon.hispidus	z+	24	25.24	0.004	3.63	2.881163	2.832873	3.558782	1	0.998	0.884
Sha	Leucanthemum.vulgare	z+	10	34.82	0.012	5.52	3.523301	2.992401	3.586091	1	0.982	0.844
Sha	Lotus.corniculatus	z+	25	38.31	0.004	9.01	3.11763	3.059702	3.402918	1	1	1
Sha	Luzula.campestris	z+	7	27.07	0.008	5.99	3.483079	2.961076	3.558782	0.978	0.9	0.736
Sha	Oxalis.stricta	z+	73	52.11	0.004	4.01	2.857332	2.702443	3.483892	1	0.996	0.934
Sha	Peucedanum.oreoselinum	z+	19	27.69	0.004	4.72	3.304166	2.859523	3.523301	1	0.996	0.866
Sha	Picris.hieracioides	z+	6	20.66	0.004	7.66	3.304166	3.186621	3.586091	1	0.978	0.914
Sha	Plantago.lanceolata	z+	52	42.87	0.008	3.84	2.801589	2.700846	3.586091	1	0.994	0.844
Sha	Potentilla.erecta	z+	5	11.9	0.004	5.45	3.173921	3.141648	3.586091	0.99	0.938	0.78
Sha	Potentilla.reptans	z+	69	58.07	0.016	2.9	2.493973	2.475302	3.48061	0.974	0.962	0.728
Sha	Primula.acaulis	z+	16	36.15	0.004	8.61	3.215075	3.165815	3.586091	1	1	0.998
Sha	Ranunculus.bulbosus	z+	27	29.67	0.004	4.45	2.832873	2.82894	3.523301	1	1	0.946
Sha	Rubus.fruticosus	z+	13	28.15	0.004	7.59	3.186621	3.096203	3.499337	1	1	0.994
Sha	Rumex.acetosa	z+	62	57.37	0.004	5.63	2.801589	2.618868	3.128085	1	1	0.996
Sha	Rumex.acetosella	z+	26	64.17	0.004	6.31	3.586091	2.930629	3.586091	0.98	0.924	0.724
Sha	Salvia.pratensis	z+	10	22.83	0.004	5.9	3.304166	3.077118	3.483079	1	0.99	0.942
Sha	Sanguisorba.minor	z+	11	21.7	0.004	5.83	3.196284	2.999982	3.499337	1	0.994	0.944
Sha	Setaria.viridis	z+	7	13.73	0.004	4.69	3.109219	3.052328	3.391257	0.996	0.97	0.86
Sha	Silene.pratensis	z+	34	34.93	0.004	5.89	3.156752	2.908002	3.427332	1	1	0.982
Sha	Silene.vulgaris	z+	45	46.18	0.004	7.99	3.067371	2.862786	3.398321	1	1	1
Sha	Thalictrum.minus	z+	19	24.05	0.008	4.67	2.911117	2.867863	3.558782	1	1	0.94
Sha	Thymus.pulegioides	z+	14	24.85	0.004	7.1	3.156752	2.999982	3.483079	1	1	0.968
Sha	Trifolium.pratense	z+	35	41.75	0.004	5.75	3.258308	2.856857	3.558782	1	1	0.964
Sha	Urtica.dioica	z+	38	40.69	0.004	6.77	2.988469	2.911117	3.087675	1	1	0.992
Sha	Veronica.chamaedrys	z+	16	19.06	0.004	3.54	3.165815	2.859523	3.434386	1	0.988	0.794
Sha	Vicia.angustifolia	z+	40	62.52	0.008	4.33	3.523301	2.817775	3.586091	0.994	0.99	0.866
Sha	Vicia.cracca	z+	28	33.98	0.004	6.44	3.031588	2.898805	3.434431	1	1	0.992
Sha	Vicia.sepium	z+	15	18.99	0.008	3.7	2.911117	2.862786	3.499337	1	0.992	0.81
Sha	Vincetoxicum.hirundinaria	z+	8	20.51	0.004	7.51	3.192117	3.128085	3.523301	1	0.996	0.954
Sha	Viola.sp.	z+	25	29.51	0.008	4.12	3.240165	2.930629	3.48509	1	0.984	0.878

Table 4:A5 List of indicator species for high and mid-to-high values of biodiversity in Swiss vineyards. For each biodiversity measure, we report the specificity value (A), fidelity (B), IndVal value and the statistical significance of the association (p val).

#	**Biodiversity measure**	**Sampling plot group**	**Species**	**A**	**B**	**IndVal**	**p value**
1	FRic	M+H	Galium.mollugo	0.67234	0.55294	0.81	0.001
2	FRic	M+H	Erigeron.annuus	0.64814	0.56471	0.782	0.001
3	FRic	M+H	Arrhenatherum.elatius	0.65681	0.44706	0.763	0.001
4	FRic	M+H	Oxalis.stricta	0.5048	0.54118	0.735	0.015
5	FRic	M+H	Convolvulus.arvensis	0.57387	0.50588	0.699	0.014

#	Biodiversity measure	Sampling plot group	Species	A	B	IndVal	p value
6	FRic	M+H	Achillea.millefolium	0.69206	0.47059	0.685	0.003
7	FRic	M+H	Crepis.capillaris	0.70244	0.47059	0.674	0.011
8	FRic	M+H	Vicia.angustifolia	0.63058	0.30588	0.671	0.001
9	FRic	M+H	Rumex.acetosa	0.51191	0.45882	0.67	0.045
10	FRic	M+H	Holcus.lanatus	0.56982	0.43529	0.661	0.005
11	FRic	M+H	Anthoxanthum.odoratum	0.53769	0.34118	0.641	0.002
12	FRic	M+H	Silene.vulgaris	0.50819	0.31765	0.62	0.014
13	FRic	H	Bromus.sterilis	0.72753	0.47619	0.589	0.001
14	FRic	H	Daucus.carota	0.85089	0.40476	0.587	0.001
15	FRic	H	Silene.pratensis	0.63774	0.52381	0.578	0.005
16	FRic	M+H	Urtica.dioica	0.67487	0.31765	0.57	0.013
17	FRic	H	Hedera.helix	0.81187	0.38095	0.556	0.002
18	FRic	H	Lotus.corniculatus	0.71807	0.40476	0.539	0.003
19	FRic	H	Brachypodium.pinnatum	0.70669	0.38095	0.519	0.002
20	FRic	H	Rubus.fruticosus	0.90153	0.2619	0.486	0.001
21	FRic	H	Cruciata.glabra	0.79773	0.28571	0.477	0.001
22	FRic	H	Sanguisorba.minor	0.9337	0.2381	0.471	0.001
23	FRic	H	Lathyrus.pratensis	0.72354	0.28571	0.455	0.002
24	FRic	H	Veronica.chamaedrys	0.72818	0.2619	0.437	0.008
25	FDiv	M+H	Taraxacum.officinale	0.6114	0.8816	0.877	0.001
26	FDiv	M+H	Veronica.persica	0.7399	0.6184	0.716	0.035
27	FDiv	M+H	Poa.trivialis	0.63	0.5	0.699	0.007
28	FDiv	M+H	Cerastium.fontanum	0.6038	0.4605	0.662	0.009
29	FDiv	M+H	Plantago.major	0.5424	0.2763	0.569	0.003
30	FDiv	M+H	Glechoma.hederacea	0.594	0.2763	0.563	0.014
31	FDiv	M+H	Bellis.perennis	0.5605	0.2237	0.558	0.001
32	FDiv	M+H	Ranunculus.repens	0.5352	0.25	0.533	0.01
33	FDiv	H	Hordeum.murinum	0.6959	0.3095	0.464	0.028
34	FDiv	H	Poa.annua	0.6201	0.3095	0.438	0.043
35	FDiv	H	Euphorbia.helioscopia	0.6967	0.2619	0.427	0.017
36	RaoQ	M+H	Veronica.persica	0.72588	0.53086	0.826	0.001
37	RaoQ	M+H	Geranium.molle	0.60874	0.62963	0.793	0.002
38	RaoQ	M+H	Stellaria.media	0.70237	0.61728	0.781	0.001
39	RaoQ	M+H	Digitaria.sanguinalis	0.63044	0.65432	0.761	0.005
40	RaoQ	H	Cardamine.hirsuta	0.69899	0.61538	0.656	0.002
41	RaoQ	M+H	Cerastium.fontanum	0.71006	0.48148	0.653	0.023
42	RaoQ	M+H	Lamium.purpureum	0.70243	0.37037	0.626	0.006
43	RaoQ	H	Capsella.bursa.pastoris	0.62102	0.53846	0.578	0.007
44	RaoQ	H	Portulaca.oleracea	0.60695	0.38462	0.483	0.01
45	Ric	M+H	Galium.mollugo	0.59957	0.57143	0.804	0.001
46	Ric	M+H	Arrhenatherum.elatius	0.67795	0.46429	0.793	0.001
47	Ric	M+H	Erigeron.annuus	0.74279	0.58333	0.789	0.001
48	Ric	M+H	Oxalis.stricta	0.53321	0.60714	0.761	0.003

#	Biodiversity measure	Sampling plot group	Species	A	B	IndVal	p value
49	Ric	M+H	Crepis.capillaris	0.69464	0.47619	0.745	0.001
50	Ric	M+H	Achillea.millefolium	0.64834	0.42857	0.737	0.001
51	Ric	M+H	Rumex.acetosa	0.52329	0.47619	0.736	0.001
52	Ric	M+H	Holcus.lanatus	0.63393	0.46429	0.735	0.001
53	Ric	M+H	Potentilla.reptans	0.49925	0.5119	0.712	0.015
54	Ric	M+H	Silene.vulgaris	0.59831	0.34524	0.69	0.001
55	Ric	M+H	Anthoxanthum.odoratum	0.63867	0.35714	0.672	0.001
56	Ric	M+H	Plantago.lanceolata	0.59417	0.39286	0.653	0.006
57	Ric	M+H	Urtica.dioica	0.54678	0.25	0.625	0.001
58	Ric	H	Daucus.carota	0.79046	0.37778	0.546	0.001
59	Ric	H	Primula.acaulis	0.86862	0.31111	0.52	0.001
60	Ric	H	Rubus.fruticosus	0.85865	0.26667	0.479	0.001
61	Ric	H	Cruciata.glabra	0.85223	0.26667	0.477	0.002
62	Ric	H	Lathyrus.pratensis	0.72097	0.24444	0.42	0.025
63	Ric	H	Thymus.pulegioides	0.71973	0.24444	0.419	0.009
64	Sim	M+H	Galium.mollugo	0.67817	0.44	0.85	0.001
65	Sim	M+H	Arrhenatherum.elatius	0.66848	0.4	0.81	0.001
66	Sim	M+H	Erigeron.annuus	0.65129	0.52	0.803	0.001
67	Sim	M+H	Achillea.millefolium	0.65839	0.36	0.763	0.001
68	Sim	M+H	Crepis.capillaris	0.65227	0.41333	0.755	0.001
69	Sim	M+H	Rumex.acetosa	0.51996	0.44	0.752	0.001
70	Sim	M+H	Holcus.lanatus	0.50384	0.37333	0.748	0.001
71	Sim	M+H	Silene.vulgaris	0.60884	0.30667	0.716	0.001
72	Sim	M+H	Anthoxanthum.odoratum	0.66213	0.32	0.681	0.001
73	Sim	H	Cruciata.glabra	0.87619	0.52381	0.677	0.001
74	Sim	H	Daucus.carota	0.80119	0.57143	0.677	0.001
75	Sim	M+H	Plantago.lanceolata	0.63668	0.37333	0.666	0.003
76	Sim	M+H	Artemisia.verlotiorum	0.69052	0.32	0.596	0.047
77	Sim	H	Primula.acaulis	0.81907	0.42857	0.592	0.001
78	Sim	H	Brachypodium.pinnatum	0.69665	0.47619	0.576	0.001
79	Sim	M+H	Hypochaeris.radicata	0.62521	0.26667	0.567	0.006
80	Sim	H	Trifolium.pratense	0.54591	0.57143	0.559	0.014
81	Sim	H	Peucedanum.oreoselinum	0.62258	0.42857	0.517	0.004
82	Sim	H	Salvia.pratensis	0.85301	0.28571	0.494	0.001
83	Sim	H	Lathyrus.pratensis	0.82711	0.28571	0.486	0.004
84	Sim	H	Rubus.fruticosus	0.71864	0.28571	0.453	0.004
85	Sha	M+H	Galium.mollugo	0.68795	0.53465	0.826	0.001
86	Sha	M+H	Arrhenatherum.elatius	0.72325	0.46535	0.803	0.001
87	Sha	M+H	Erigeron.annuus	0.69789	0.58416	0.773	0.001
88	Sha	M+H	Crepis.capillaris	0.67902	0.48515	0.744	0.001
89	Sha	M+H	Rumex.acetosa	0.50968	0.48515	0.741	0.001
90	Sha	M+H	Achillea.millefolium	0.68947	0.45545	0.736	0.001
91	Sha	M+H	Oxalis.stricta	0.61567	0.61386	0.733	0.024

#	Biodiversity measure	Sampling plot group	Species	A	B	IndVal	p value
92	Sha	M+H	Holcus.lanatus	0.6746	0.45545	0.728	0.001
93	Sha	M+H	Silene.vulgaris	0.6535	0.35644	0.702	0.001
94	Sha	M+H	Anthoxanthum.odoratum	0.74806	0.37624	0.665	0.003
95	Sha	M+H	Urtica.dioica	0.49343	0.25743	0.653	0.001
96	Sha	M+H	Silene.pratensis	0.67601	0.28713	0.599	0.004
97	Sha	H	Lotus.corniculatus	0.78872	0.4186	0.575	0.002
98	Sha	M+H	Hypochaeris.radicata	0.58807	0.26733	0.554	0.012
99	Sha	M+H	Trifolium.pratense	0.69606	0.29703	0.553	0.023
100	Sha	H	Primula.acaulis	0.8759	0.30233	0.515	0.005
101	Sha	H	Cruciata.glabra	0.85548	0.27907	0.489	0.007
102	Sha	H	Thymus.pulegioides	0.79224	0.27907	0.47	0.007
103	Sha	H	Rubus.fruticosus	0.77039	0.27907	0.464	0.006
104	Sha	H	Carex.caryophyllea	0.75218	0.25581	0.439	0.017

Table 4:A6 List of 52 indicators species associated to high and mid-to-high values of taxonomic (**Ric**hness, **Sim**pson and **Sha**nnon) and functional (**F**unctional **Ric**hness, **F**unctional **Div**ergence and **Rao**) biodiversity measures. **Occ**: indicates for how many biodiversity indices each species was significant.

		Functional indices			Taxonomic indices		
Indicator species	**Occ**	**FRic**	**FDiv**	**Rao**	**Ric**	**Sim**	**Sha**
Achillea millefolium	4	x			x	x	x
Anthoxanthum odoratum	4	x			x	x	x
Arrhenatherum elatius	4	x			x	x	x
Crepis capillaris	4	x			x	x	x
Cruciata glabra	4	x			x	x	x
Erigeron annuus	4	x			x	x	x
Galium mollugo	4	x			x	x	x
Holcus lanatus	4	x			x	x	x
Rubus fruticosus	4	x			x	x	x
Rumex acetosa	4	x			x	x	x
Silene vulgaris	4	x			x	x	x
Daucus carota	3	x			x	x	
Lathyrus pratensis	3	x			x	x	
Oxalis stricta	3	x			x		x
Urtica dioica	3	x			x		x
Brachypodium pinnatum	2	x				x	
Lotus corniculatus	2	x					x
Silene pratensis	2	x					x
Primula acaulis	3				x	x	x

		Functional indices			Taxonomic indices		
Indicator species	**Occ**	**FRic**	**FDiv**	**Rao**	**Ric**	**Sim**	**Sha**
Thymus pulegioides	2				x		x
Hypochaeris radicata	2					x	x
Trifolium pratense	2					x	x
Plantago lanceolata	2				x	x	
Cerastium fontanum	2		x	x			
Veronica persica	2		x	x			
Bromus sterilis	1	x					
Convolvulus arvensis	1	x					
Hedera helix	1	x					
Sanguisorba minor	1	x					
Veronica chamaedrys	1	x					
Vicia angustifolia	1	x					
Bellis perennis	1		x				
Euphorbia helioscopia	1		x				
Glechoma hederacea	1		x				
Hordeum murinum	1		x				
Plantago major	1		x				
Poa annua	1		x				
Poa trivialis	1		x				
Ranunculus repens	1		x				
Taraxacum officinale	1		x				
Lamium purpureum	1			x			
Geranium molle	1			x			
Capsella bursa pastoris	1			x			
Cardamine hirsuta	1			x			
Digitaria sanguinalis	1			x			
Portulaca oleracea	1			x			
Stellaria media	1			x			
Potentilla reptans	1				x		
Artemisia verlotiorum	1					x	
Peucedanum oreoselinum	1					x	
Salvia pratensis	1					x	
Carex caryophyllea	1						x

Chapter 5 Comment évaluer la qualité botanique des surfaces agricoles de promotion de la biodiversité? L'agroécosystème viticole au Sud des Alpes suisses comme cas d'étude

L'un des vignobles de l'étude, à Camorino (TI).

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Résumé

En Suisse, l'Ordonnance sur les paiements directs régule le versement des contributions pour la biodiversité des surfaces agricoles. La qualité écologique est estimée sur la base de plantes indicatrices et de structures de valeur particulière. Toutefois, l'instrument pour sélectionner les indicateurs permettant de mesurer la qualité botanique fait défaut. Dans un travail réalisé en 2008 et 2011, nous proposons un cadre conceptuel qui définit quatre critères pour la sélection d'espèces indicatrices: 1) Intensité de gestion, 2) Composantes de la biodiversité, 3) Vulnérabilité et danger d'extinction, et 4) Dommage réel ou potentiel pour la biodiversité. Appliqué aux vignobles au Sud des Alpes suisses, cet outil a permis de sélectionner au total 118 espèces indicatrices associées positivement à de basses intensités de gestion, à de hauts niveaux de biodiversité, à une augmentation du risque d'extinction ou à une menace elevée pour la biodiversité.

Summary

The Ordinance on direct payments in Switzerland regulates the payments of subsidies for biodiversity in agricultural surfaces. The ecological quality is estimated through the assessment of indicator plant species and particularly valuable structures. However, a tool for the selection of suitable indicators to measure botanical quality is missing. With the present work, which was carried out in 2008 and 2011, we propose a conceptual framework defining four criteria for the selection of indicator plant species: 1) Management intensity, 2) Components of biodiversity, 3) Vulnerability and threat of extinction, 4) Real and potential harm to biodiversity. Applying the framework to the vineyards of Southern Switzerland allowed to select a total of 118 species. These where associated with low management intensities, high biodiversity levels, increased threat of extinction, and a high degree of harm to biodiversity.

Keywords direct payments, indicator species; agri-environment measures, ecological performance, biodiversity.

Introduction

Le rapport de l'Évaluation des écosystèmes pour le millénaire (EM 2005) souligne la relation importante qui existe entre les services fournis par les écosystèmes, la biodiversité, le bien-être et la santé de l'homme. Différentes études, notamment, ont quantifié la perte de services écosystémiques due à la perte de biodiversité (p. ex. Bastian 2013; Harrison *et al*. 2014). Dans les agroécosystèmes, l'ensemble des organismes associés aux plantes cultivées supporte des services d'importance primordiale, comme le recyclage des nutriments et la régulation des organismes nuisibles (Altieri et Nicholls 2004). Les champs cultivés sont caractérisés par un apport constant d'éléments externes dont l'intensification conduit souvent à un appauvrissement de la diversité biologique - et donc à la perte de services écosystémiques (Lucas *et al*. 2013; Power 2010).

L'instrument des paiements pour les services écosystémiques (PSE) est utilisé en agriculture pour prévenir ce risque et promouvoir des externalités positives (Ferraro et Kiss 2002; Milne et Niesten 2009), par exemple avec les incitations pour la promotion de la biodiversité. Pour l'octroi de telles subventions sont utilisés des indicateurs biologiques qui servent à mesurer le niveau de biodiversité d'un agroécosystème (Sommerville *et al*. 2011). La communauté scientifique a largement admis qu'il était important d'utiliser des indicateurs reflétant différentes composantes de la biodiversité (p. ex. Devictor *et al*. 2010; Trivellone *et al*. 2014), qui fournissent des informations complémentaires sur les services écosystémiques (Perronne *et al*. 2014). Plus les indicateurs couvrent différents aspects de la biodiversité, sur le plan taxonomique (richesse et diversité spécifique, espèces rares) ou sur le plan fonctionnel (richesse et diversité fonctionnelle), et plus les stratégies agro-environnementales sont efficaces (de Bello *et al*. 2010; Mace et Baillie 2007).

En Suisse, l'ordonnance sur les paiements directs (Office fédéral de l'agriculture, 23 octobre 2013) régule le versement des contributions pour la biodiversité en faveur de 16 types de surfaces qui répondent à des niveaux de qualité déterminés. La qualité écologique des surfaces est estimée à travers des plantes indicatrices et des structures de valeur particulière. Par conséquent, la sélection de ces espèces est fondamentale pour l'évaluation correcte de la qualité des surfaces de promotion de la biodiversité. Toutefois, en l'état actuel des choses, un instrument pour la sélection appropriée de tels indicateurs fait défaut.

La présente contribution souhaite proposer un cadre conceptuel qui définisse les critères de sélection d'espèces indicatrices de la qualité botanique sur les surfaces de promotion de la biodiversité. Nous proposons, par ailleurs, une méthode de sélection des espèces basée à la fois sur des analyses quantitatives et sur l'évaluation d'experts. L'agroécosystème viticole au Sud des Alpes de la Suisse est utilisé ici comme cas d'étude. En conclusion, les résultats sont confrontés aux exigences relatives à l'art. 59 et à l'annexe 4 sur les surfaces viticoles présentant une biodiversité naturelle.

Matériel et méthodes

Cadre conceptuel

Le cadre d'une sélection d'espèces indicatrices doit être appliqué à des surfaces agricoles pour la promotion de la biodiversité, situées dans une région homogène sur le plan biogéographique et socio-culturel. Le choix de l'unité géographique de référence suit la division de la Suisse en régions biogéographiques proposée par l'Office fédéral de l'environnement (OFEV) (Gonseth *et al*. 2001). Dans chacune de ces régions, la sélection des espèces indicatrices nécessite de réaliser des relevés floristiques représentatives de l'ensemble de la région considérée.

Le cadre est fondé sur 4 principaux critères de sélection, indépendants l'un de l'autre et divisés en étapes (fig. 1). Chaque critère génère une sous-liste d'espèces indicatrices; la liste totale s'obtient en additionnant les sous-listes, sachant qu'une espèce peut être sélectionnée selon un ou plusieurs critères.

• **Critère 1 - Intensité de gestion**, divisé en trois étapes: 1a) sélection de zones homogènes du point de vue de la végétation et définition du type et de l'intensité de gestion appliqués; 1b) sélection d'un seuil d'intensité de gestion pour chaque zone identifiée. Celui-ci permet de mettre les relevés floristiques effectués sur chaque type de zone dans deux groupes, associés respectivement à une basse et une haute intensité de gestion; 1c) sélection des espèces indicatrices associées aux basses intensités de gestion.

• **Critère 2 - composantes de la biodiversité**, lui aussi divisé en trois étapes: 2a) sélection d'une ou plusieurs composantes de la biodiversité à considérer (p. ex. génétique, taxonomique et fonctionnelle) et, pour chacune d'elles, d'un ou plusieurs indices de biodiversité. Ces indices seront appliqués aux données des relevés des parcelles échantillons; 2b) sélection d'un seuil pour chaque indice, qui permet de mettre les relevés floristiques effectués sur chaque type de zone dans deux groupes, associés respectivement à de bas et de hauts niveaux de biodiversité; 2c) sélection des espèces indicatrices associées aux hauts niveaux de biodiversité.

• **Critère 3 - évaluation de la vulnérabilité et danger d'extinction des espèces**, divisé en 2 étapes: 3a) sélection des espèces menacées d'extinction ou vulnérables dans la région considérée selon la Liste Rouge des espèces menacées de Suisse (Moser et al. 2002) en utilisant la liste complète des espèces relevées dans les parcelles échantillons; 3b) choix d'espèces indicatrices d'intérêt spécifique pour le type de surface agricole considéré et qui peuvent justifier une intervention de sauvegarde et de protection.

• **Critère 4 - dommage réel ou potentiel pour la biodiversité causé par des espèces particulières**, divisé en 2 étapes: 4a) sélection des espèces qui causent, actuellement ou potentiellement, des dommages à la diversité biologique, la santé et/ou l'économie et dont l'expansion doit être empêchée ou surveillée, selon la Liste Noire, «Watch List» (http://www.infoflora.ch/fr/flore/neophytes/listes-et-fiches.html) ou d'autres sources bibliographiques; 4b) sélection des espèces qui, dans le type de surface agricole considéré, indiquent un appauvrissement et une banalisation de la végétation.

Les espèces indicatrices selon les Critères 1 et 2 (fig.1) sont sélectionnées en analysant l'ensemble de la communauté des espèces; par conséquent, les relevés floristiques doivent être de type quantitatif (couverture ou abondance des différentes espèces). Les espèces indicatrices des Critères 3 et 4 (fig.1) sont en revanche évaluées à partir de la liste complète des espèces et sur des données de présence/absence d'un relevé de type qualitatif.

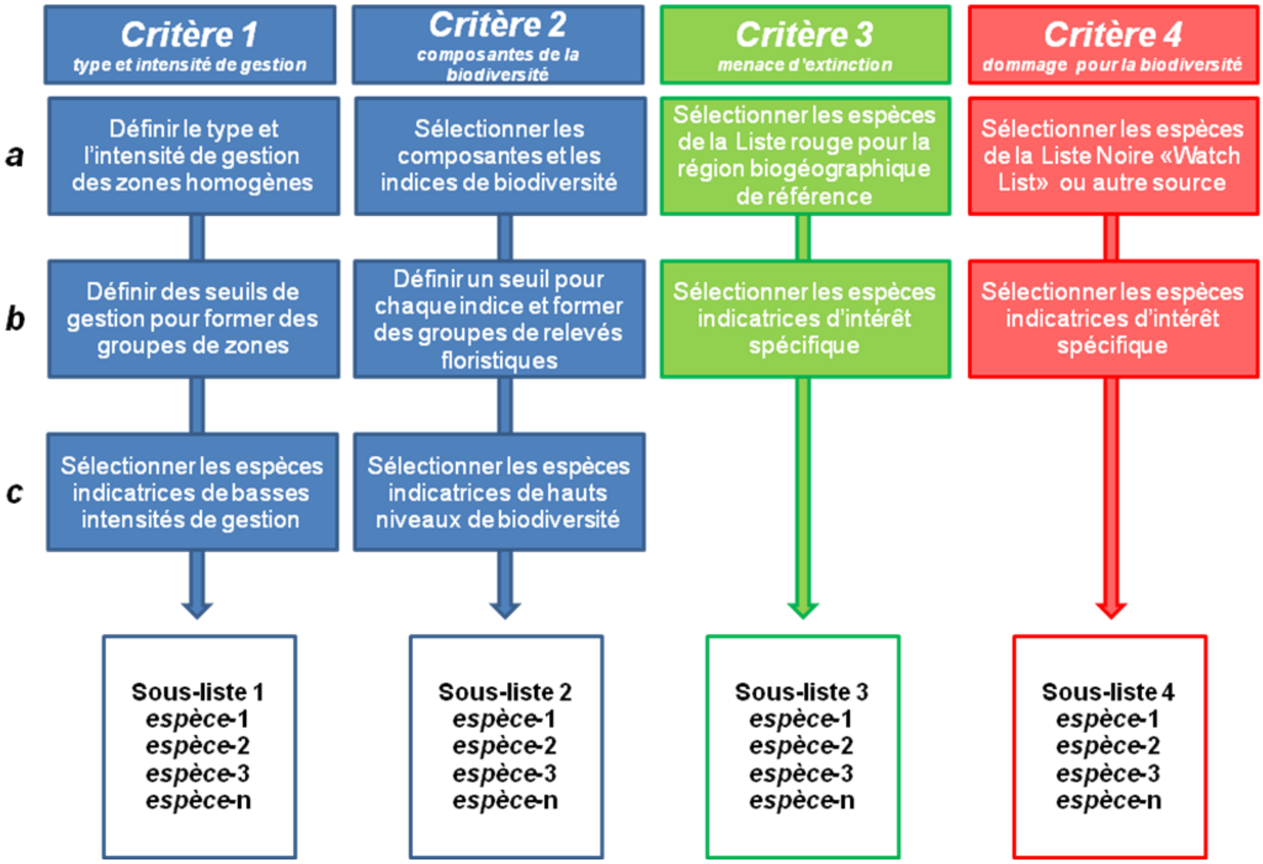

Figure 5:1 Cadre conceptuel pour la sélection des espèces indicatrices de qualité botanique des surfaces agricoles de promotion de la biodiversité, avec 4 critères indépendants divisés en étapes (a-c). Les Critères 1 et 2 (en bleu) sélectionnent les espèces indicatrices de communautés végétales de grande valeur écologique, le Critère 3 (en vert) sélectionne les espèces en danger d'extinction et le Critère 4 (en rouge) les espèces dangereuses (Liste Noire) ou potentiellement dangereuses (Watch List) pour la santé, l'économie et la biodiversité. La liste totale est obtenue en additionnant les sous-listes 1-4, une espèce pouvant être sélectionnée par un ou plusieurs critères.

Cas d'étude: l'agroécosystème viticole au sud des Alpes suisses

Relevés floristiques

L'agroécosystème viticole au Sud des Alpes de la Suisse se situe dans la région biogéographique SA (Gonseth *et al*. 2001). La flore a été relevée dans 48 vignobles échantillons (fig. 2, points rouges) sélectionnés en fonction de la pente, de l'exposition et de la composition du paysage (dans un rayon de 500 m autour des vignobles). Les relevés quantitatifs ont été effectués dans chaque vignoble d'après la méthode de Londo (1976). La couverture des différentes espèces a été estimée en considérant 5 carrés de 1 m^2 sur chaque zone homogène identifiée. Les relevés qualitatifs de présence/absence des espèces ont été réalisés dans les 48 vignobles échantillons et dans 33 autres vignobles (fig. 2, points marrons), soit 81 vignobles au total. La liste complète des espèces a été établie en parcourant l'ensemble de la surface plantée de vignes, zones de manœuvre comprises. La nomenclature des espèces est celle de Lauber *et al*. (2012). Les relevés ont été effectués en 2008 (fin juin) et en 2011 (à la fin du printemps et en été). Les données relatives à la gestion ont été recueillies dans des questionnaires aux viticulteurs.

Analyse des données

Des analyses statistiques multivariées ont été appliquées aux données des relevés quantitatifs. Les seuils d'intensité de gestion ont été définis notamment par analyse multi-variée MRT (Multiple Regression Tree) (De'ath 2002). L'analyse multi-variée TITAN (Threshold Indicator Taxa ANalysis) (Baker et King 2010) a servi à définir les seuils des valeurs de biodiversité, qui correspondent chacun à un changement significatif de diversité et/ou de composition des espèces. Ces méthodes sont plus amplement décrites dans Trivellone *et al*. (2014).

La biodiversité taxonomique et la biodiversité fonctionnelle ont été considérées dans cette étude. Parmi les indices de biodiversité taxonomique nous avons utilisé le nombre d'espèces, l'indice de Simpson et l'indice de Shannon; alors que pour la diversité fonctionnelle on a considéré l'indice de Richesse fonctionnelle, la Divergence fonctionnelle e la Diversité fonctionnelle de Rao (pour une synthèse, voir Magurran et McGill 2011). Ces indices sont assez largement utilisés, solides et reconnus pour fournir des informations complémentaires sur la structure des communautés et sur les aspects liés à la résilience fonctionnelle des écosystèmes. Tous les indices ont été calculés en fusionnant les données des relevés des 5 carrés de chaque zone homogène. Les seuils identifiés par analyses MRT et TITAN ont servi à former des groupes de zones semblables. Les groupes sont utilisés pour sélectionner les espèces indicatrices à l'aide d'analyses IndVal (Indicator Value analysis) (De Cáceres *et al*. 2010). Seules les espèces indicatrices associées à une basse intensité de gestion et à de hauts niveaux de biodiversité ont été retenues pour la liste finale.

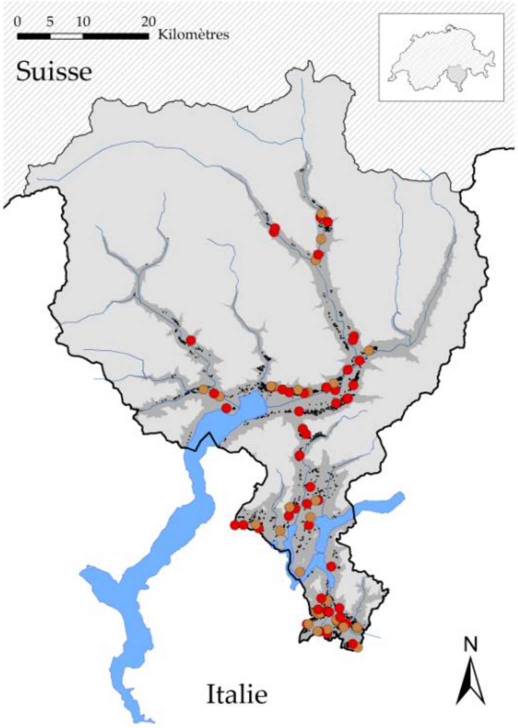

Figure 5:2 Situation des 81 vignobles sélectionnés dans la région biogéographique du Sud des Alpes suisses pour l'application du cadre conceptuel (voir Fig. 5:1).
Les relevés quantitatifs ont été effectués dans 48 vignobles (points rouges), 33 vignobles supplémentaires (points bruns) ont été choisis pour le relevé qualitatif.

Résultats et discussion

Dans les 81 vignobles étudiés en 2008 et 2011, 520 espèces au total appartenant à 281 genres et 91 familles ont été relevées. Elles représentent 18 % de la flore de la région biogéographique SA et 15 % de la flore suisse.

Seules 10 espèces (dont *Trifolium repens*, *Plantago lanceolata*, *Erigeron annuus* et *Stellaria media*) sont ubiquistes et figurent dans plus de 73 vignobles, tandis que 269 espèces ont été relevées dans moins de 5 vignobles, tels *Aphanes australis*, *Ornithogalum umbellatum* (fig. 3), *Torilis arvensis* et *Arum italicum*. Une étude de la flore relevée dans 31 vignobles de Suisse romande (Clavien et Delabays 2006) montre une structure similaire des communautés. Toutefois, parmi les 10 espèces les plus répandues, seul *T. repens* figure dans les deux études.

Figure 5:3 L'ornithogale en ombelle (*Ornithogalum umbellatum*), une espèce assez rare dans le vignoble au Sud des Alpes.

Le cadre conceptuel appliqué aux données

L'application du cadre conceptuel au donnés quantitatives et qualitatives fourni les résultats suivants:

Critère 1a: dans la région biogéographique SA, trois types de zone homogènes ont été identifiés à l'intérieur des vignobles: le rang (espace sous les pieds des vignes d'une largeur de 50 cm), l'interligne (espace entre deux rangs) et le talus (espace incliné séparant un ou plusieurs rangs et interlignes). La couverture végétale dans ces trois zones peut être gérée par le désherbage et par la fauche. Le désherbage est le principal type de gestion sur le rang, avec au maximum 3 applications par an d'herbicides systémiques, la fauche étant généralement réservée à l'interligne et au talus, avec au maximum respectivement 7 et 4 fauchages par an. Le tableau 5:1 rassemble les résultats sur la typologie et l'intensité de gestion.

Tableau 5:1 Typologies et régimes de gestion principaux du rang, de l'interligne et des talus dans les vignobles au Sud des Alpes suisses. Nb= quantité totale de zones, Min= valeur minimum d'intensité de gestion observée, Max= valeur maximum d'intensité de gestion observée.

Gestion	Zone	Nb	Min	Max
Désherbage[1]	Rang	48	0	3
Fauchage[2]	Interligne	48	2	7
	Talus	24	1	4

[1] Exprimé en applications d'herbicide par année.
[2] Exprimé en fauches par année

Critère 1b: les analyses MRT ont marqué les seuils de gestion suivants: aucune application annuelle d'herbicide sur le rang (0, tabl. 5:2), trois fauches par an de l'interligne au maximum (3, tabl. 5:2) et deux fauches par an pour le talus (2, tabl. 2). Selon ces seuils, les relevés floristiques de chaque zone ont été répartis dans les groupes à haute ou à basse intensité de gestion.

Critère 1c: l'analyse IndVal a sélectionné des espèces indicatrices pour chacun des groupes susmentionnés. Pour une basse intensité de gestion, 35 espèces ont été identifiées comme indicatrices (p. ex. *Arrhenatherum elatius*, *Anthoxanthum odoratum* et *Brachypodium pinnatum*). Le tableau 5:2 présente quelques-unes des espèces indicatrices de la sous-liste 1.

Tableau 5:2 Espèces indicatrices d'une basse intensité de gestion (seuils de gestion entre crochets) sélectionnées pour chaque zone (rang, interligne, talus) à l'intérieur du vignoble et en fonction du Critère 1 du cadre conceptuel (Analyse IndVal, valeur de P: * = 0,01; ** = 0,001)

Rang	**Interligne**	**Talus**
Nb. d'applications d'herbicide/an **[0]**	**Nb. de fauches/an** **[≤3]**	**Nb. de fauches/an** **[≤2]**
*Urtica dioica*****	*Arrhenatherum elatius*****	*Brachypodium pinnatum******
*Galium mollugo*****	*Anthoxanthum odoratum*****	*Daucus carota*****
*Rumex acetosa*****	*Clinopodium vulgare***	*Carex caryophyllea*****

Seules quelques espèces sélectionnées sont indiquées à titre d'exemple. Pour la liste complète, contacter le premier auteur.

Critère 2a: les indices de diversité taxonomique et fonctionnelle ont été calculés pour chaque zone échantillon. Par exemple, pour la composante taxonomique, la richesse en espèces varie d'un minimum de 10 espèces sur le rang à un maximum de 61 espèces sur l'interligne. Les données détaillées sont disponibles auprès du premier auteur.

Critère 2b: l'analyse TITAN appliquée aux valeurs des indices de biodiversité a fourni des seuils permettant de répartir les relevés floristiques de chaque zone en deux groupes: bas et hauts niveaux de biodiversité.

Critère 2c: l'analyse IndVal a sélectionné des espèces indicatrices pour chacun des groupes susmentionnés. Au total 43 espèces indicatrices sont associées à des hautes niveaux de biodiversité sur le rang (dont *Galium mollugo* et *Veronica persica*), 49 espèces sur l'interligne (dont *Achillea millefolium* et *A. elatius*) et 30 sur le talus (dont *A. millefolium* et *B. pinnatum*). Le tableau 5:3 présente quelques-unes des espèces indicatrices de la sous-liste 2.

Tableau 5:3 Espèces indicatrices de hauts niveaux de biodiversité taxonomique et/ou fonctionnelle sélectionnées pour chaque zone (rang, interligne, talus) à l'intérieur du vignoble et en fonction du Critère 2 du cadre conceptuel (Analyse IndVal, valeur de P : *= 0.01; **= 0.001)

Rang	**Interligne**	**Talus**
Niveaux élevés de biodiversité taxonomique et/ou fonctionnelle		
Galium mollugo**	Arrhenatherum elatius**	Achillea millefolium**
Veronica persica**	Anthoxanthum odoratum**	Brachypodium pinnatum*
Lamium purpureum**	Achillea millefolium**	Silene vulgaris*

Seules quelques espèces sélectionnées sont indiquées à titre d'exemple. Pour la liste complète, contacter le premier auteur.

Critère 3a: des 520 espèces recensées, 43 (8,3 %) sont menacées d'extinction, fortement menacées ou vulnérables dans la région biogéographique SA, selon la Liste Rouge suisse.

Critère 3b: parmi ces 43 espèces, 7 sont particulièrement liées aux milieux agricoles (Delarze et Gonseth 2008) et entrent dans la sous-liste 3. Parmi elles, les espèces ségétales *Scleranthus annuus* (un vignoble sur 81 étudiés) et *Torilis arvensis* (trois vignobles) et les adventices *Misopates orontium* (un vignoble) et Veronica agrestis (trois vignobles). Par ailleurs, les rares populations d'*A. italicum* et *Aristolochia rotunda* présentes dans la région biogéographique SA sont souvent liées aux vignobles.

Critère 4a: des 520 espèces recensées, 17 (3,3 %) figurent sur la Liste Noire et la Watch List.

Critère 4b: toutes les espèces relevées sont inclues dans la sous-liste 4 car elles constituent une menace réelle ou potentielle pour la santé, l'économie et la biodiversité.

Au total, les sous-listes obtenues selon le cadre conceptuel recensent 118 espèces pour les vignobles de la région SA: 35 espèces sélectionnées pour le Critère 1, 57 pour le Critère 2, 9 pour le Critère 3 et 17 pour le Critère 4. Certaines des espèces indicatrices de basses intensités de gestion et de hauts niveaux de biodiversité sont caractéristiques de prairies de fauche de basse altitude, prairies sèches, forêts mésophiles, ourlets maigres ou zones rudérales (Delarze et Gonseth 2008), parmi lesquelles *A. millefolium*, *A. elatius* et *Silene vulgaris* pour les prairies de fauche sur sols modérément humides et riches en nutriments; *A. odoratum* et *Cerastium fontanum* qui résistent à une fauche modérée (jusqu'à deux fois/an). D’autres espèces, comme *Carex caryophyllea*, *Daucus carota* et *B. pinnatum*, dominent dans des prairies semi-arides et sont considérées sensibles au fauchage (Briemle et Ellenberg 1994). Les résultats de cette étude montrent que l’écosystème viticole n’est pas un habitat exclusif pour les espèces de la Liste Rouge. Leur présence dans la vigne semble plutôt fortuite et due à la colonisation des milieux environnants ou à une présence antérieure à la plantation de la vigne. Certaines espèces toutefois sont liées aux agroécosystèmes en général (Delarze et Gonseth 2008) ou au vignoble et sont pour cette raison proposées dans la sous-liste du Critère 3.

Selon les instructions de l’art. 59 et de l’annexe 4 de l’Ordonnance sur les paiements directs dans l’agriculture (Office fédéral de l'agriculture, janvier 2014), les contributions de niveau de qualité II sont accordées aux surfaces viticoles pour leur biodiversité naturelle lorsqu'une certaine valeur écologique est dépassée, basée sur un inventaire floristique, une liste des espèces particulières et des structures d'une valeur particulière. A chaque espèce est attribué un nombre de points indiquant sa valeur écologique. Dans la liste actuelle des espèces particulières, une importance considérable est accordée aux espèces menacées d'extinction en Suisse ou dans une région biogéographique donnée, en leur attribuant un nombre de points très élevé. Même si ce principe est souvent appliqué dans les programmes de protection de la biodiversité (Vandewalle *et al*. 2010), la communauté scientifique reconnaît que les espèces vulnérables sont souvent trop rares pour être les seules qui importent dans la définition de la qualité écologique (Rosenthal 2003; Zechmeister *et al*. 2003). Les Critères 1 et 2 permettent de sélectionner des espèces indicatrices de basse intensité de gestion et de hauts niveaux de biodiversité, qui révèlent la présence de communautés végétales de grande valeur écologique dans la région SA (voir les tableaux 5:2 et 5:3). Ces espèces devraient être intégrées dans la liste pour l'évaluation de la qualité botanique des vignobles et revêtir davantage d’importance par rapport aux espèces menacées d'extinction. Les espèces sélectionnées à travers le Critère 3, en revanche, doivent être jugées comme d'une grande valeur intrinsèque parce qu’elles sont menacées d'extinction et donc rares, mais devraient faire l'objet de contributions ciblées pour être spécifiquement protégées. Elles doivent de toute manière être intégrées dans la liste des espèces particulières à côté des espèces sélectionnées selon les Critères 1 et 2. Les espèces sélectionnées dans le Critère 4 représentent une menace pour la biodiversité. Toutefois, dans les vignobles de la région SA, les néophytes ont peu de chances de se développer du fait que les activités de gestion de la couverture végétale contribuent à leur contrôle. Comme elles peuvent néanmoins constituer une source de diffusion vers les milieux environnants, il conviendrait de les insérer dans la liste des espèces particulières, mais avec un nombre négatif de points. Le but est d'encourager le viticulteur à lutter ponctuellement contre ces plantes particulières.

L'application du cadre proposé permet d'obtenir des valeurs-seuil utiles pour définir des niveaux de gestion à faible intensité qui perturbent peu la végétation associée à la culture. De plus, une importance appropriée est accordée à deux composantes principales de la biodiversité (taxonomique et fonctionnelle), dans le but de préserver à la fois la richesse spécifique et le fonctionnement de l'écosystème.

Le cadre conceptuel proposé permet de sélectionner des espèces indicatrices à travers un système rigoureux et scientifiquement reproductible. Par ailleurs, sa portée dépasse l'essai présenté ici, puisqu'il permet de choisir les aspects de la biodiversité auxquels donner plus de poids, ce qui le rend polyvalent et transposable à d'autres agroécosystèmes.

Conclusions

Dans cette étude, un cadre conceptuel est proposé pour sélectionner des espèces indicatrices de qualité botanique dans des surfaces agricoles de promotion de la biodiversité. Ses points forts sont les suivants:

- il est spécifique pour des régions biogéographiques homogènes;
- il est basé sur des critères de sélection et des analyses quantitatives reproductibles;
- il intègre différentes composantes de la biodiversité qui se complètent entre elles;
- il tient compte des pratiques de gestion propres à la région biogéographique de référence;
- il est applicable aux autres typologies de surfaces agricoles de promotion de la biodiversité.

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Chapter 6 A regional-scale survey to define the known and potential vectors of grapevine yellows phytoplasmas in vineyards South of Swiss Alps

Scaphoideus titanus Ball, 1932 vector of phytoplasma causing Flavescence dorée disease (photo: V. Trivellone)

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Abstract

The most important Grapevine Yellows (GY) phytoplasma diseases in Europe are Flavescence dorée (FD) and Bois noir (BN); they are spread in vineyard by two proven vectors, *Scaphoideus titanus* Ball and *Hyalesthes obsoletus* Signoret, respectively. Other potential vectors of GY have been identified, which are thought to play a secondary role. The GY control strategies are not always effective and an in-depth study on the ecological cycle of the pathogens at regional scale, would be of paramount importance. This study was carried out in 48 representative sites of wine-growing area South of Swiss Alps, with the aim to identify known and potential vectors and to characterize the FD and BN phytoplasmas isolates. Out of 167 Auchenorrhyncha species recorded, 27 were known or potential vectors of phytoplasmas and five of those tested positive for phytoplasmas. *S. titanus* was infected by 16SrV-D subgroup phytoplasma and no clear relationship between its population density and disease outbreaks was observed. *Orientus ishidae* harboured 16SrV-C and 16SrV-D subgroups suggesting its potential role in spreading 16SrV-C phytoplasma isolates from arboreal plants to grapevine, and FD-D from grapevine to grapevine. *H. obsoletus* was infected by BN phytoplasmas, *tuf*-types a and b, however it was collected with relatively low abundance. *Reptalus panzeri* and *R. cuspidatus* tested positive to *tuf*-type b, but only *R. cuspidatus* was common and abundant in the investigated vineyards. To define the range of alternative vectors using a detailed approach on regional scale provides background information to get a clearer vision of the spread of phytoplasmas in vineyards.

Keywords Auchenorrhyncha, grapevine yellows, molecular detection, vectors.

Introduction

Grapevine yellows (GY) are severe diseases occurring in *Vitis vinifera* in all wine growing countries. They cause very serious damage in viticulture and wine industry, ranging from a lower yield of berries and wine to the progressive decline and death of the infected plants. The etiological agents of GY are phytoplasmas, belonging to the new-established genus '*Candidatus* Phytoplasma' (*Ca*. P.) that includes at least 37 different species and phylogenetic groups (IRPCM 2004; Marcone 2014). Only some phytoplasma species occur in grapevine worldwide, causing similar but distinct diseases.

In Europe, two phytoplasmas are typically present in grapevine: phytoplasmas belonging to the 16SrV-C and 16SrV-D phylogenetic subgroups, causing *Flavescence dorée* (FD), and '*Ca*. P. solani' (stolbur phytoplasma), belonging to the 16SrXII phylogenetic group and associated to Bois noir (BN). FD is a quarantine disease in the European Community, discovered in France in the '70s and nowadays occurring in several countries of Southern and Central Europe (EPPO 2007). Different FD phytoplasma isolates were identified in grapevine, such as FD70, FD-D (=FD92) and FD-C, they are transmitted from vine to vine by *Scaphoideus titanus* Ball (Schvester et al. 1963), a nearctic leafhopper that spends its whole life cycle on *Vitis* spp. and disseminates FD phytoplasmas in an epidemical manner. BN phytoplasma is transmitted by the occasional grapevine-feeder *Hyalesthes obsoletus* Signoret that usually lives on weeds (Sforza *et al*. 1998; Maixner 1994). BN is spread in Europe and in the Mediterranean Basin, where it generally shows an endemic behaviour. Indeed '*Ca*. P. solani' usually infects only a low percentage of the plants in vineyard, due to the different ecological cycle of the pathogen. According to the genetic sequences of the elongation factor Tu, two main '*Ca*. P. solani' types can be distinguished, *tuf*-type a and *tuf*-type b, which are involved in two different epidemiological cycles mainly related to *H. obsoletus* (Langer and Maixner 2004). *Tuf*-type a is associated to *Urtica dioica* and *tuf*-type b to *Convolvulus arvensis*. Other known and potential vectors of GY have been recognized in Europe, which are thought to play a secondary role in GY epidemiology. In Germany, the alder-feeding Hemiptera *Oncopsis alni* (Schrank) (Maixner and Reinert 1999) was demonstrated to transmit PGY (Palatinate Grapevine Yellows) phytoplasma, similar to FD phytoplasma, from the black alder, *Alnus glutinosa,* to grapevine (Maixner et al. 2000). In Italy and Serbia the polyphagous planthopper *Dictyophara europaea* (Linnaeus) was shown to be able to transmit FD phytoplasma from wild clematis, *Clematis vitalba*, to grapevine (Filippin *et al*. 2009). *Orientus ishidae* (Matsumura) was found infected by FD and FD-related phytoplasmas in a few countries, thus it is considered a suspected vector (Mehle *et al*. 2010; Gaffuri *et al*. 2011). Naturally infected *Reptalus panzeri* (Low) specimens were able to transmit '*Ca.* P. solani' to grapevine in Serbia (Cvrković *et al*. 2014), and the same phytoplasma was identified in other *Reptalus* species (Pinzauti *et al*. 2008; Palermo *et al*. 2004; Mikec *et al*. 2006). At last, other leafhopper species inhabiting vineyard agroecosystems were found to harbour '*Ca.* P. solani', such as *Euscelis lineolatus* Brullé*, Anaceratagallia ribauti* (Ossiannilsson) and *Macrosteles quadripunctulatus* (Kirschbaum), but their ability to transmit BN phytoplasma to grapevine has not been confirmed (Batlle *et al*. 2008; Riedle-Bauer *et al*. 2008; Landi *et al*. 2013).

GY control strategies are mainly focused on prevention, survey and insecticide treatments, which are not always effective. Concerning FD, despite mandatory control programs against *S. titanus* and grubbing of diseased vineyards, the contaminated surfaces increase every year, suggesting that the epidemiology of the diseases is still not completely understood. In the case of BN, there are no established and effective control strategies, as the insecticide treatments are useless in vineyards, due to the fact that the vector feeds only occasionally on grapevine. Indeed, occurrence of BN has increased over the last few years in most European countries (Johannesen *et al*. 2008).

Against this background, an in-depth study on the ecological cycle of the pathogens, together with the research of alternative vectors and their role in GY outbreaks, would be of paramount importance, because the epidemiological context could be quite different in diverse countries.

In Switzerland BN and FD diseases have been recorded in 2001 (Schmid and Emery 2001) and 2004 (Schaerer *et al*. 2007), respectively; however, the epidemiological aspects are still poorly understood. BN is quite widespread in all wine-growing regions, while FD occurs only South of the Swiss Alps. A number of actions have already been undertaken in order to cope with the diffusion of known vectors; nevertheless, so far, outbreaks of GY are still observed. The objectives of the present work were: 1) to identify known and potential vectors by means of a detailed screening of leafhoppers inhabiting southern Swiss vineyards; 2) to assess the presence of FD and BN phytoplasmas in the insects captured in GY infected vineyards; 3) to characterize the phytoplasma isolates infecting leafhoppers. In this context, we also discuss the use of an experimental design based on a representative subset of samples to address phytoplasma disease issues. To this aim, a representative sample of vineyards South of the Swiss Alps was surveyed for the Auchenorrhyncha and phytoplasma occurrence, in order to gain more insight and to lay the groundwork for further investigations.

Materials and methods

Study area and experimental design

The study area comprises the whole wine-growing area of southern Switzerland which extends from the northernmost site Ludiano (46°25'N – 8°58'E) to the southernmost site Pedrinate (45°49'N – 9°00'E), ranging from 199 m to 589 m a.s.l.

Forty-eight study sites were selected using a design that accounted for the three main variables characterizing the vineyard agroecosystem of the study region, i.e. aspect (24 sites were exposed SE-SW; 24 sites NE-NW), slope (24 sites were on a plain <5°; 24 sites were terraced >10°) and the dominant land use type (>50% land unit cover) surrounding the vineyard within a radius of 500 m (16 sites were dominated by forest, 16 by settlements, 16 by open areas). The 48 selected vineyards could be considered representative of the vineyard ecosystem and landscape of southern Switzerland (Appendix 6:A1).

Since 2004, a surveillance monitoring was launched by the phytosanitary office in southern Switzerland in order to oversee the spread of phytoplasma diseases (Jermini *et al*. 2014). Out of 48 investigated vineyards, five were affected by FD, 13 by BN, 10 by both and 20 showed no GY symptoms in the last decade. A chitin synthesis inhibitor insecticide (Buprofezin) was applied twice per year (on June) in 26 studied vineyards (Appendix 6:A1).

In the study area, the vineyard floor is usually permanently covered by wild native plants, e.g. *Trifolium* spp., *Plantago lanceolata*, *Stellaria media*, *Erigeron annuus*, *Ranunculus* spp. (for further details on the floristic composition of vineyards of this study see Trivellone *et al*. 2014). In some vineyards, the vine rows could be tilled or treated with herbicides (a strip of around 50 cm of width below the grapevines).

Collection and faunistic data evaluation

In 2011, multiple sampling methods were used to effectively pick up the greatest number of specimens and species of leafhoppers. Four standard methods were selected: D-Vac sampler, pitfall traps, beating tray and yellow sticky traps. The first two were used for capturing leafhoppers related to the herb layer and to the soil surface, the last two for the species inhabiting the vine canopy. Vineyards were sampled from April to October, for a total of seven sampling periods. In each vineyard two sampling sites were placed which consisted of a pitfall trap and a sticky trap, one site along a vine row and the other in a vegetated embankment (in terraced vineyards) or along another vine row (in lowland vineyards). The two sampling sites were at least 20 m apart and 20 m away from vineyard margin to avoid edge effects; the traps were open for seven days per month in each sampling period. The pitfall trap site consisted in four 200 ml cups (7 cm of diameter) recessed into the soil and arranged along a vine row or embankment, and spaced about 1 m one another. Each cup was half-filled with saline solution and covered by a transparent plastic roof. The yellow sticky trap (Rebell®, 15×8 cm dimensions) was hung inside the vine canopy at about 1.5 m above the ground. By means of D-vac sampler and beating tray, two samples were collected monthly from two transects inside the vineyard. The D-Vac sampler was applied on ground cover vegetation during 120 seconds per sample. Beating tray (sheet opening 1×1m) was applied to collect all arthropods that fell down from vine canopy after shaking of thirty grapevines per sample. Additional sampling sites were placed along the vineyard-forest ecotone in a total of 16 vineyards (Appendix 6:A1).

All collected leafhopper specimens were identified to species level, then preserved in 70% alcohol and maintained at -20°C. Nomenclature followed Ribaut (1936), Della Giustina (1989) and Holzinger *et al*. (2003).

Sample-based rarefaction curve was calculated using vineyard as the sample to evaluate sampling adequacy to detect the regional (gamma) species richness (Gotelli and Colwell 2001).

After a literature review and field data evaluation, a sub-group of Auchenorrhyncha species known or potential vectors of phytoplasmas were selected for the investigated region; the relationship between species mean abundance and species occurrence was examined and the specimens were subjected to molecular analyses.

DNA extraction and amplification

Each sample consisted of a pool of up to 20 specimens of the same species. Total DNA was extracted according to Gatineau *et al*. (2001).

TaqMan real time PCR analysis on ribosomal genes was carried out to identify the presence of the 16SrV and 16SrXII group phytoplasmas, according to the protocols already described (Angelini *et al*. 2007). The assays were performed in 96-well plates on a CFX96 thermal cycler (Biorad). Positive samples were then amplified by nested PCR on three different DNA fragments for a deeper genetic characterization. '*Ca*. P. solani' positive samples were analysed in the *16S-23S ribosomal RNA*, *tuf* and *secY* nucleotide regions. FD phytoplasmas positive samples were screened in the *16S-23S rRNA*, *rplV-rpsC* and *secY* regions.

In the *16S-23S rRNA* region, the first PCR was performed using universal primer pair P1/P7 and the nested PCR with primers 16r758f/M23Sr (Angelini *et al*. 2001).

FD phytoplasmas positive samples were amplified in the *rplV-rpsC* region, encoding for ribosomal proteins L22 and S3, in nested PCR using primers rp(V)F1/rpR1, followed by rp(V)F1A/rp(V)R1A (Martini *et al*. 2002; Lee *et al*. 2004). PCR assays on the *secY* gene, encoding the preprotein translocase subunit SecY, were performed with primers FD9f2/FD9r, followed by FD9f3/FD9r2 (Angelini *et al*. 2001). The concentrations of reagents and PCR conditions for amplification of *16S-23S rRNA*, *rplV-rpsC* and *secY* regions were as described in Angelini *et al*. (2001) and Martini *et al*. (2002).

Characterization of '*Ca*. P. solani' was performed in the *tuf* gene using primers fTuf1/rTuf1, followed by fTufAy/rTufAy, according to Schneider *et al*. (1997). *Tuf* gene encodes for elongation factor Tu. For the *secY* gene of '*Ca*. P. solani' positive samples, new primers were designed, using the *rplO-secY-adk* sequences of three phytoplasmas phylogenetically close to '*Ca.* P. solani': two '*Ca.* Phytoplasma asteris' isolates (AYWB Aster yellows witches'-broom phytoplasma, GenBank accession number CP000061, and OY-M Onion yellows phytoplasma, GenBank accession number AP006628) and one '*Ca*. Phytoplasma australiense' isolate (GenBank accession number AM422018). This genomic region codifies for the 50S ribosomal protein L15, the preprotein translocase subunit SecY and the Adenylate kinase. The forward primer RPLOf2 (5'-CAA AGA ATT CCT AAA AGA GG-3') and the reverse ADKr2 (5'-GCT TGA GTG CCT TTG CCA ATT CC-3') were used for the direct PCR, whereas the pair RPLOf3 (5'-TCT ATT TTA GCA GTT GGT GG-3') and BN9r0 (5'-AAA CTT GTT CCT CCT AAT TTC-3') was used for the nested PCR. The reaction mixture contained 1 µl of extracted DNA or of the diluted first PCR product (1:50) as template, 0.3 mM each dNTP, 0.6 µM each primer, 0.75 U Taq DNA polymerase (Sigma Aldrich) and the buffer supplied with the enzyme. The $MgCl_2$ concentrations were 3 mM and 1.5 mM in the direct and nested PCR, respectively. The following thermal protocol was used for both amplifications: initial denaturation at 94 °C for 3', then 40 cycles of 1' at 94 °C, 2' at 48 °C and 3' at 66 °C, and a final extension for 7' at 66 °C. The final PCR products are 1202 bp long.

Amplicons were visualized on 1% agarose gel stained with GelRed (Biotium Inc.) under a GelDoc XR UV transilluminator (Biorad).

RFLP analysis and DNA sequencing

Aliquots of the nested PCR products obtained from the *16S-23S rRNA* and *tuf* genomic regions were digested with restriction enzymes, according to the manufacturer's instructions. The

16r758f/M23Sr fragments were processed using *Taq*I (MBI Fermentas), the fTufAy/rTufAy fragments using *Hpa*II (MBI Fermentas). The restriction patterns were compared with those of FD-D, FD-C and '*Ca*. P. solani' phytoplasmas for *16S-23S rRNA* amplicons, and with a *tuf*-type a and a *tuf*-type b BN phytoplasmas for *tuf* ones. Restriction products were separated by 10% polyacrylamide gel electrophoresis in TBE buffer, stained with GelRed and visualized with GelDoc XR.

All amplicons were purified with Sephadex G-100 (GE Healthcare). Quantification was carried out with a ND-100 Spectrophotometer (NanoDrop Technologies Inc.). Sequencing was performed with the BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems) and the products were purified with Sephadex G-50 (Sigma-Aldrich). Samples were finally loaded into an automatic ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Sequence analysis

Electropherograms were corrected and aligned using CLUSTAL W with BioEdit 7.0.9. Phylogenetic trees were constructed with the Maximum Parsimony (MP), Minimum Evolution (ME) and Neighbor Joining (NJ) methods using the MEGA 5.0 software package. The reliability of the analyses was subjected to a bootstrap test with 1000 replicates. Reference sequences were selected from GenBank. A BLAST query was previously performed in order to select the most similar reference sequences. Nucleotide sequences obtained in this study were deposited in the DDJB/EMBL/GenBank databases under accession numbers KP635226-KP635235, KT310178 (for BN *secY* gene), KR350639-KR350642, KT371524- KT371527 (for FD *secY* gene), KP890031, KP941109, KP941110, KR024255-KR024261(for *16S-23S rRNA* region), KR029136-KR029140 (for *tuf* gene) and KR350643-KR350645, KT371528- KT371531 (for *rplV-rpsC* region) (Appendix 6:A2).

Results

Occurrence of Auchenorrhyncha known and potential vectors

In total, 60 936 specimens belonging to 167 Auchenorrhyncha species were recorded from the 48 sites across four sampling methods (Appendix 6:A3). Among them, there were 39 Fulgoromorpha species from the families Cixiidae (5), Delphacidae (28), Dictyopharidae (1), Flatidae (1), Issidae (2), Tettigometridae (2), and 128 Cicadomorpha species from the families Aphrophoridae (5), Cercopidae (2), Membracidae (3), Ulopidae (1), Cicadellidae (117). The appendix 6:A4 illustrates the rarefaction curve based on the number of sampled vineyards for species richness of Auchenorrhyncha fauna: the curve reached an asymptote well before total accumulated sampling effort, suggesting that species inventory was complete in this study.

After a literature review, a total of 22 species out of 167 recorded were allocated to the status of known or putative vectors of phytoplasmas and then suspected to be involved in the spread of phytoplasmas in vineyards in southern Switzerland. After field data evaluation, five further species (i.e. *Arocephalus longiceps*, *Centrotus cornutus*, *Issus coleoptratus*, *Japananus hyalinus* and *Penthimia nigra*) were selected and allocated to the status of suspected vectors in this study. These species were considered because are widespread in vineyards where outbreaks of GY were observed and no presence of known or potential vectors, according to literature, was recorded (Appendix 6:A5). Overall, 27 species were considered for molecular analyses: six of them (*A. ribauti*, *A. longiceps*, *Macrosteles cristatus*, *Psammotettix confinis*, *Reptalus cuspidatus* and *S. titanus*) were widespread throughout the study area with highest population densities (occurrence > 24 and mean abundance > 60); five species (*Aphrodes makarovi*, *Euscelis incisus*, *H. obsoletus*, *Megophthalmus scanicus* and *Philaenus spumarius*) were also quite widespread but with relatively lower mean abundance (occurrence > 34 and mean abundance < 30). *Macrosteles viridigriseus* was much less widespread, but abundant (if not dominant) when present (occurrence = 12 and mean abundance = 100). Most species (15 as a total) only occurred in vineyard at very low mean abundance and were less widespread (occurrence ≤ 20 and mean abundance ≤ 20) (Appendix 6:A6).

Phytoplasma detection and RFLP analyses

PCR analysis was performed on 3 529 specimens belonging to 27 species, pooled in 371 samples (Appendix 6:A7). Phytoplasma DNA was detected in 19 samples out of 371 (5.1%), collected from eight vineyards out of 48 (17%), corresponding to five species: *O. ishidae, S. titanus, H. obsoletus, R. cuspidatus* and *R. panzeri*.

The FD and FD-related phytoplasmas were harboured by two out of 149 (1.3%) collected *S. titanus* samples and six out of 22 (27%) collected *O. ishidae* samples. The RFLP analyses of the *16S-23S rRNA* genetic region showed that *O. ishidae* and *S. titanus* harboured FD-related phytoplasmas;

notably, all isolates from *S. titanus* samples and one from *O. ishidae* belonged to 16SrV-D subgroup, while the other samples from *O. ishidae* belonged to 16SrV-C phylogenetic subgroup (Table 6:1).

The stolbur phytoplasma was harboured by eight *H. obsoletus* samples out of 39 (21%) collected, two *R. cuspidatus* samples out of 28 (7%) and one *R. panzeri* out of seven (14%). *Hpa*II digestion of *tuf* amplicons showed a prevalence of *tuf*-type a in phytoplasmas from *H. obsoletus* (seven on eight tested positive samples), while one phytoplasma isolate from *H. obsoletus* and all isolates from *Reptalus* spp. were *tuf*-type b (Table 6:1).

Table 6:1 Results of phytoplasma strain differentiation by RFLP analyses on *16S-23S rRNA* and *tuf* amplicons for leafhopper samples tested positive in this study. Details about vineyards are reported in Appendix 6:A1.

			Local scale	**Regional scale**
Insect species	**Phytoplasma type**	**Vineyard**	**positive/tested (%)**	**positive/tested (%)**
Scaphoideus titanus	16SrV-D	Camorino	1/4 (25)	2/149 (1)
		Stabio	1/1 (100)	
Orientus ishidae	16SrV-D	Stabio	1/7 (14)	6/22 (27)
	16SrV-C	Stabio	3/7 (43)	
		Lamone	1/2 (50)	
		Rancate	1/1 (100)	
Hyalesthes obsoletus	16SrXII-A (*tuf*-type a)	Rancate	1/2 (50)	8/39 (21)
		Croglio	4/10 (50)	
		Porza	1/1 (100)	
		Camorino	1/2 (50)	
	16SrXII-A (*tuf*-type b)	Rovio	1/7 (14)	
Reptalus panzeri	16SrXII-A (*tuf*-type b)	Besazio	1/1 (100)	1/7 (14)
Reptalus cuspidatus	16SrXII-A (*tuf*-type b)	Rovio	1/3 (33)	2/28 (7)
		Croglio	1/5 (20)	

Multilocus sequence typing analyses on FD phytoplasma

To further characterize the detected phytoplasmas, a multilocus sequence typing (MLST) was performed. Different phylogenetic trees were created for each genetic region. All the trees were constructed using the MP method; the NJ and ME trees generally showed the same topology (data not shown). The *16S-23S rRNA*, *rplV-rpsC* and *secY* regions from FD and FD-related phytoplasmas were sequenced, obtaining a total of 23 sequences.

16S-23S rDNA - Data from the *rRNA* genetic fragments always confirmed the RFLP patterns. All 16SrV-D sequences were identical to one another and to the FD-D reference strain (AJ548787), as expected. Also all 16SrV-C sequences were identical to one another and to the reference strains (AF458378; AF176319; AF458379; Y16387).

rplV-rpsC - The phylogenetic tree based on 769 bp of the *rplV-rpsC* region defined four main groups, each encompassing members of widely used reference strains: FD70, FD-D, FD-C, and ALY (Appendix 6:A8). The sequence obtained from the sample Oi-63 was not included in this phylogenetic tree because it showed many nucleotide ambiguities, suggesting the presence of at least two distinct 16SrV isolates. In the first group, three 16SrV-C phytoplasma isolates harboured by *O. ishidae* (Oi-46, Oi-370 and Oi-78) showed a sequence identity of 100% with the French FD70 reference strain, CL-AL31 isolate from an Italian clematis and V04-11-55 isolate from a French grapevine. The phytoplasma isolate from one *O. ishidae* sample (Oi-369) showed a single point mutation with respect to FD70; however, it sub-grouped with samples previously collected from grapevines in Northern Italian regions Valle d'Aosta (Vv-AO262) and Piedmont (VI04-248-04). In the second group, the Swiss 16SrV-D phytoplasma isolates were all identical to the FD-D reference isolate isolated from *V. vinifera* in Italy. The last two groups encompassed reference strains only, represented by FD-C and ALY respectively, which were included just to show the phylogenetic distance from the samples of the present study.

secY - The phylogenetic tree based on 1 004 bp of *secY* gene (Fig. 6:1) showed a similar overall topology to that inferred from the ribosomal protein genes. Some further differentiations are highlighted in the first group where Oi-370, Oi-46 and Oi-78 are slightly different from the FD70 reference strain. In the second group, the three samples Oi-368, St-371, St-182 again showed a 100% sequence identity with that of FD-D reference strain isolated from *O. ishidae* in Slovenia.

As a total, three vineyards hosted populations of *O. ishidae* infected by the three different isolates of FD and FD-related phytoplasmas. In particular, four out of six *O. ishidae* samples testing positive in this study were collected in a vineyard (Stabio) very close to the border with Lombardy (Italy) where the detected *O. ishidae* population, inhabiting the forest surrounding the vineyard, harboured three different FD variants, belonging to 16 SrV-C and D phylogenetic subgroups. The two *S. titanus* samples (St-371 and St-182), harbouring the same identical isolate, were collected in two different vineyards far away to each other and the insecticides were applied only in one of them (Stabio).

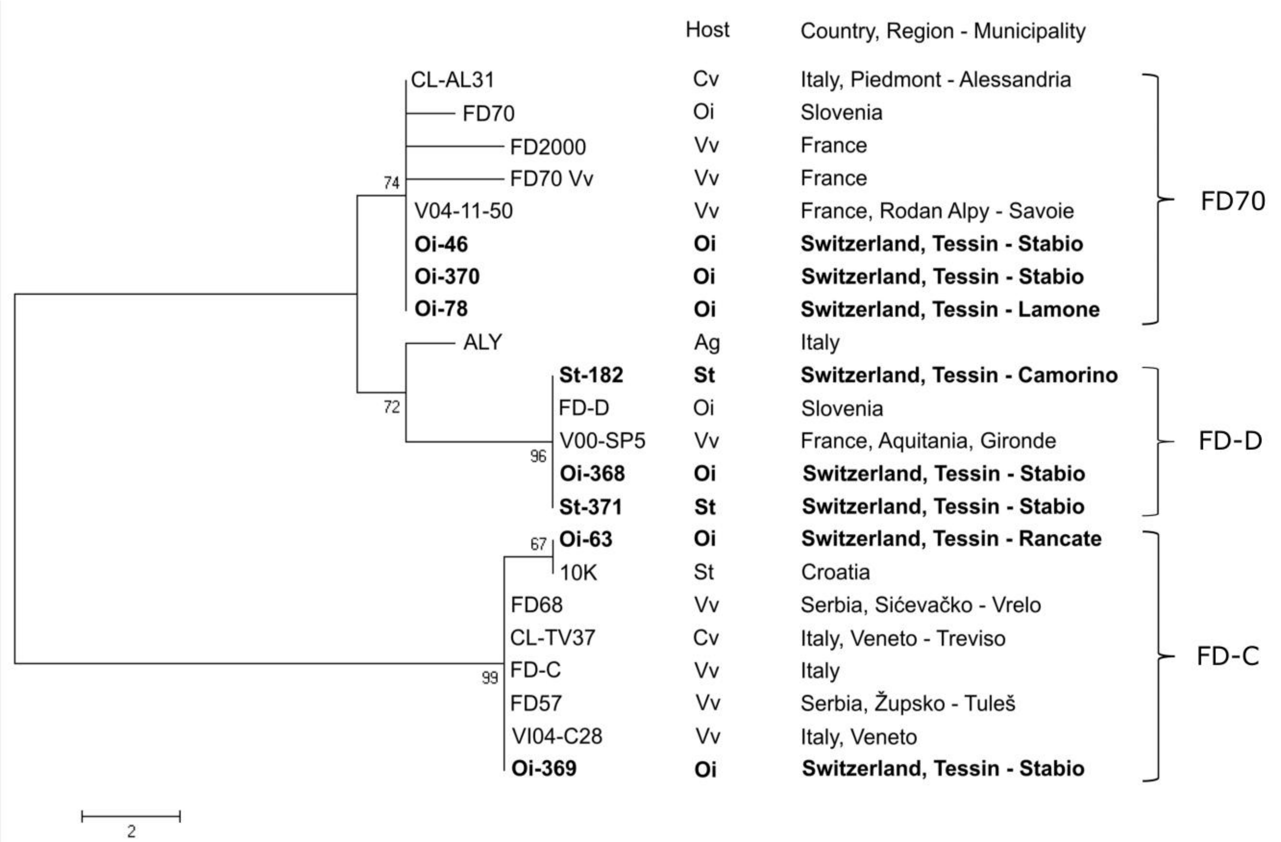

Figure 6:1 Phylogenetic tree based on 1 004 bp of the *secY* gene sequences obtained from *Scaphoideus titanus* and *Orientus ishidae* samples. Swiss samples processed in the current study are evidenced in bold. Bar length is proportional to the number of base substitutions per site. Numbers on the branches are confidence values obtained for 1 000 replicates. GenBank accession numbers and details of the reference phytoplasma strains are listed in Appendix 6:A2. Host: Ag *Alnus glutinosa*; Cv *Clematis vitalba*; Oi *Orientus ishidae*; St *Scaphoideus titanus*; Vv *Vitis vinifera*.

In Table 6:2 an overview of FD phytoplasma isolates detected in this study is reported.

MLST analyses on stolbur phytoplasma

A MLST analysis was performed on the detected stolbur phytoplasmas. Different phylogenetic trees were created for each genetic region. All the trees were constructed using the MP method; the NJ and ME trees generally showed the same topology (data not shown). The *16S-23S rRNA*, *tuf* and *secY* nucleotide fragments of stolbur phytoplasmas were sequenced, for a total of 33 nucleotide sequences.

16S-23S rDNA - The sequence data of *rRNA* genomic fragments always confirmed the RFLP patterns. The newly obtained sequences were very similar to one another, showing two or three single nucleotide polymorphisms compared to '*Ca*. P. solani' type strain (AF248959) (data not shown).

tuf - The phylogenetic tree based on 826 bp of the *tuf* gene allowed distinguishing two main groups that correspond to *tuf*-type a and *tuf*-type b restriction patterns (Appendix 6:A9). All *H. obsoletus* but one (Ho-98) were infected by *tuf*-type a, whereas *Reptalus* species by *tuf*-type b. The sequences of the Swiss samples were identical to one another and to those of many other isolates from grapevines, weeds and insects collected in Central-Eastern Europe. In the *tuf*-type b cluster, the two *R. cuspidatus* sequences showed identity with the Austrian isolate CrHo12-650 and the Macedonian ones HoU17 and HoU93; their *tuf*-type was named "b2" by Aryan *et al*. (2014) and "ab" by Atanasova *et al*. (2015). *H. obsoletus* (Ho-98 sample) and the *R. panzeri* (Rp-202 sample) harboured a *tuf*-type b1 corresponding to the classical *tuf*-type b according to Aryan *et al*. (2014).

secY - Characterization of the more variable *secY* gene allowed a finer distinction among the stolbur isolates, that clustered in any case according to their RFLP *tuf*-type. Three different sequence profiles were identified in all the *H. obsoletus* samples infected by *tuf*-type a phytoplasma, clearly separated in the phylogenetic tree based on 677 bp from *secY* amplicons (Fig. 6:2). In the first one (seq-a), four phytoplasmas isolates from *H. obsoletus* samples shared the same nucleotide identity and they were identical with isolates harboured by *V. vinifera* and *U. dioica* sampled in North Western and North Eastern Italy, respectively. A second sequence (seq-b) comprised just two *H. obsoletus* samples (Ho-167 and Ho-263) and in the third sequence profile (seq-c) one *H. obsoletus* sample (Ho-69) showed a 100% nucleotide identity with a *V. vinifera* sample from Italy. The two *R. cuspidatus* samples confirmed to harbour identical isolates of '*Ca.* P. solani' and were included in the seq-d profile. In the last sequence profile (seq-e), one *H. obsoletus* sample showed a 100% sequence identity with the only one *R. panzeri* sample testing positive in this study, the French STOLC reference strain from *Lycopersicum esculentum* and STOL11, the classical reference strain of '*Ca.* P. solani'.

In total, five vineyards hosted populations of *H. obsoletus* infected by stolbur phytoplasma and in all sites *C. arvensis* and *U. dioica* were always detected mixed inside the vineyard with the exception of Rovio where *H. obsoletus* were collected on bindweed only. The *H. obsoletus* population from Croglio harboured all the three genetic variants of *tuf*-type a, while three local populations (from Rancate, Porza and Camorino) hosted one out of three genetic variants of *tuf*-type a, and in the last population (from Rovio) a genetic variant of *tuf*-type b was detected. The two *R. cuspidatus* samples (Rc-255 and Rc-264) were collected in two different vineyards far away to each other (Rovio and Croglio), despite the phytoplasmas they harboured were identical.

In Table 6:2 an overview of BN phytoplasma strains detected in this study is reported.

Table 6:2 Overview of genetic diversity of FD and BN phytoplasma strains detected in insect bodies collected in vineyards of southern Switzerland.

Phytoplasma phylogenetic subgroup	**Strain^{a}**	**Insect species**	**Insect code**	**Locality (vineyard code)**
16SrV-D	FD-D	*S. titanus*	182, 371	Camorino (436-Caco), Stabio (778-Stab)
	FD-D	*O. ishidae*	368	Stabio (778-Stab)
16SrV-C	FD70-like	*O. ishidae*	78, 46, 370	Lamone (760-Lamo), Stabio, Stabio (778-Stab)
	FD-C/FD70-like	*O. ishidae*	369	Stabio (778-Stab)
	FD-C-like	*O. ishidae*	63	Stabio (778-Stab)
16SrXII-A	*tuf*-a, seq-a	*H. obsoletus*	112, 240, 271, 357	Rancate (912-Ranc), Porza (995-Porz), Croglio (1195-Crog), Camorino (436-Caco)
	tuf-a, seq-b	*H. obsoletus*	167, 263	Croglio (1195-Crog)
	tuf-a, seq-c	*H. obsoletus*	69	Croglio (1195-Crog)
	tuf-b, seq-e	*H. obsoletus*	98	Rovio (1095-Rovi)
	tuf-b1, seq-e (=STOL11)	*R. panzeri*	202	Besazio (796-Besa)
	tuf-b2, seq-d	*R. cuspidatus*	255, 264	Rovio (1095-Rovi), Croglio (1195-Crog)

Discussion

Since the quick outbreaks of grapevine yellows in vineyard can often persist without any clear reasons, the scientific community continues to investigate on the factors that cause these phenomena, while the stakeholders expect immediate answers and effective control strategies.

A detailed knowledge of the epidemiology of phytoplasma-associated diseases is of paramount importance; unfortunately, the epidemiologic cycles can be quite different from one another, owing to factors changing in time and space, such as phytoplasma strains, behaviour of insect vectors, primary and secondary hosts, vector population genetics and abiotic factors. In this frame, a well-designed and implemented experimental framework for the investigation of phytoplasma diseases in vineyard is desirable and advantageous to describe the particular characteristics of a territory. That being so, it is widely agreed that the first step is identifying and characterizing the phytoplasmas, since similar or closely related phytoplasmas might exhibit profound differences for host range and vectors, disease expression and development. The second crucial step is linked to the knowledge of biology of insect vectors, their occurrence, their preferred host plants and the spatio-temporal distribution in agroecosystems. The most consistent experimental approach to get objective evidences on occurrence of phytoplasmas and their carriers, according the two above-mentioned steps, is to set up investigations at regional scale, which is also the scale for which effective policy and management measures can be designed and implemented. Moreover, in order to carry out suitable studies concerning aetiology issue of phytoplasma diseases, a multidisciplinary approach is also needed.

In the present study a multidisciplinary investigation to disentangle the issues of grapevine yellows at regional scale was proposed. We collected leafhopper and planthopper samples from a representative subset of entire winegrowing area South of Swiss Alps, and the results confirmed the adequacy of sampling by means of a multi-method approach (Appendix 6:A4). As a result, a complete inventory of Auchenorrhyncha inhabiting the vineyards was provided (167 species) and the occurrence of known and potential vectors of phytoplasma was recorded. Similar studies were carried out in North Western Italy and in Austria, where the authors recorded less than one third of the species recorded in this study (32 and 57, respectively; Bosco *et al*. 1997; Kunz *et al*. 2010).

In the investigated vineyards five species tested positive for phytoplasmas: *S. titanus* and *O. ishidae,* infected by FD and FD-related phytoplasmas, and *H. obsoletus*, *R. panzeri* and *R. cuspidatus,* infected by '*Ca*. P. solani'. Among them just two species were abundant and widespread in studied area: the confirmed vector of FD phytoplasma (*S. titanus*) and a putative vector of BN phytoplasma (*R. cuspidatus*) (Appendix 6:A6). *S. titanus* was present with high occurence and abundance only in localities not subjected to mandatory control, wheras we observed very low population abundances in the other Swiss vineyards where insecticides (Buprofezin) were applied twice during the season (Appendix 6:A1). Even if a low infection rate was observed at regional scale (1%), it is worth highlighting that positive *S. titanus* samples came from a vineyard under mandatory control (one sample, St-371) and from a vineyard where insecticides had not yet been applied, nor symptoms of yellowing in grapevines observed (one sample, St-182). Whilst in Switzerland high rates of FD infected grapevine are still observed locally despite mandatory control programs, we conclude that so far there is no clear relationship between disease outbreaks and *S. titanus* population density suggesting that other leafhoppers could play a role in spreading the disease.

In Europe, *O. ishidae* was first reported in Switzerland in 2002 (Günthart and Mühlethaler 2002), while in 2010 specimens infected by FD were detected for the first time in Slovenia (Mehle *et al*. 2010) and then in Italy (Gaffuri *et al*. 2011). In this study *O. ishidae* infected by FD-related phytoplasmas was reported for the first time in Switzerland (16SrV-C and D phytoplasma isolates). Interestingly, the most common 16SrV-C phytoplasma isolate found in *O. ishidae* Swiss samples was identical with the FD isolate infecting clematis in North-West Italy (Piedmont) and grapevine in South-East France (Savoie) that are regions very close to the Swiss studied area (Fig. 1). In the investigated wine-growing area, *O. ishidae* is quite uncommon and was collected inside vineyards at low density; indeed the mosaic leafhopper inhabits broodleaved forests that surround the studied vineyards and it is strictly associated to woody plants such as *Acer, Betula, Carpinus, Crataegus, Malus, Ostrya, Salix* and so on (Hamilton,1985). For this reason, *O. ishidae* populations were detected in the vineyards where the forest is the dominant land unit in the surroundings, and most of the specimens were collected by traps placed along the borders of the forests. An high infection rate was observed both at regional (27%) and local scale (ranging from 14 to 100%). Five out of six infected *O. ishidae* samples haboured 16SrV-C subgroup phytoplasmas, a subgroup that was never recorded before in Switzerland; one sample infected by FD-D isolate was also found, which is the only phytoplasma type detected in grapevine and in *S. titanus* specimens in this region till now. All these evidences reinforced the hypothesis that *O. ishidae* could play a significant role in spreading different 16SrV-C phytoplasma isolates from arboreal plants to grapevine, and FD-D from grapevine to grapevine as well. In a next step, the presence of 16SrV-C phytoplasma isolates in grapevine and in wild plant species and the capability of *O. ishidae* to inoculate FD phytoplasmas to grapevine should be verified.

About the cixiids, *H. obsoletus* was detected with high occurrence. Previous investigations highlighted that *U. dioica* is the preferred host plant of *H. obsoletus* in most parts of Switzerland (Kessler *et al*. 2011). Afterwards, in a study on population genetic structure, Maniyar *et al*. (2013) showed that *H. obsoletus* populations from southern Switzerland were genetically very similar to those collected in Italy; and the authors highlighted the possibility for the presence of plant-unspecialized *H. oboletus* populations as already reported in Northern Italian wine-growing regions (Imo *et al*. 2013). In the present study, *H. obsoletus* was collected with relatively low abundance, because its main host can be selectively eliminated inside vineyards due to the recommended removing of the stinging nettle plants (Kehrli and Delabays 2012). In this study, locally *H. obsoletus* reached relatively high densities in vineyards where *C. arvensis* or scattered single individuals of *U. dioica* were recorded. Results reported here confirmed the presence of both *tuf*-types of BN isolates (“a” and “b”) in *H. obsoletus*, as already observed by Maniyar *et al*. (2013), and highlighted that the higher infection rates are linked with the “nettle-cycle” based on *tuf*-type a. Interestingly, in the only vineyard where the host plant of *H. obsoletus* was *C. arvensis*, the vector harboured the *tuf*-type b1.

Though four species of *Reptalus* were recorded in Europe, only *R. cuspidatus* and *R. panzeri* were detected in southern Switzerland up to now. In the studied region, *R. cuspidatus* was reported as the most common and abundant one in vineyard agroecosystems, whereas the congeneric species was rarely captured inside vineyards and it seems not to prefer grapevine. *R. panzeri* is known to be linked to *Rosa* spp. and *Prunus* spp. but also to other woody plants (*Clematis*, *Salix*, *Crataegus*, *Pinus*, and so on) (Nickel 2003); however, many authors recorded frequently this species on cultivated plants, such as grapevine and maize (Picciau *et al*. 2008; Jović *et al*. 2007). Many herbaceous dicotyledons have been recorded as host plants for *R. cuspidatus*, and specimens are frequently captured in Northestern Italian vinegrowing area on the herbaceous dicotyledons *Erodium* sp., *C. arvensis*, *Echium* sp. and *Artemisia vulgaris* (Picciau *et al*. 2008). In this study *R.*

cuspidatus was abundantly collected on ground floor inside vineyards of mixed-grass vegetation, such as *A. vulgaris*, *Geranium rotundifolium*, *Ranunculus repens* and *Trifolium* spp. Other authors have already reported some of the above mentioned herbaceous plants infected by stolbur phytoplasma, for example *A. vulgaris* (Credi *et al*. 2006) and *Trifolium pratense* (Franova *et al*. 2009). In the current study, both *R. panzeri* and *R. cuspidatus* tested positive to *tuf*-type b only, and locally they reached high infection rate, up to 100%. Based on our results, the known vector of stolbur *R. panzeri* does not seem to have an important role in the epidemiology of GY phytoplasmas in the investigated area, due to its very low population densities in vineyards. Nevertheless, the detection of a specimen positive to phytoplasma could imply a certain importance for other agroecosystems. Regarding *R. cuspidatus*, it was found to host the *tuf*-type b2 phytoplasma, previously associated to nettle cycle both in Austrian and in Macedonian vineyards (Aryan *et al*. 2014; Atanasova *et al*. 2015). Our *R. cuspidatus* specimens, however were never collected on nettle in the field. Moreover, the sequencing data showed that the *tuf*-type b2 identified here is distinct from those already characterized in nettle, *H. obsoletus* and grapevine samples in the other countries; indeed, it clustered differently in the *secY* tree compared to the previous works. This suggests that a peculiar *tuf*-type b2 occurs in Switzerland, and that other weeds may be locally infected with this stolbur isolate, that can be then acquired by *R. cuspidatus*. The range of plants hosting both *R. cuspidatus* and stolbur phytoplasma should therefore be clearly defined; moreover further investigations need to be pursued to clarify the capability of *R. cuspidatus* to inoculate *tuf*-type b phytoplasma to grapevine, as this species represents a risk factor in spreading stolbur phytoplasma in the vineyard agroecosystem.

Although the detection of a GY phytoplasma in an insect body does not necessarily prove its vector status, this detailed screening allowed us to get a more clear vision on the spreading of grapevine phytoplasmas in the vineyard agroecosystem at regional scale. This approach is very important for disentangling the complex issue of grapevine yellows, to provide background information to be used to better define the range of alternative and preferred host plants for both vectors and phytoplasmas, and to understand the possible spread of the disease. The main goal must be to develop management strategies aimed at controlling the vectors and the spreading of outbreaks.

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Appendices

Table 6:A1. Location and characteristics of the 48 investigated vineyards.

					Abiotic variables used for vineyard selection						
Municipality	**Vineyard code**	**Longitude**	**Latitude**	**Altitude (m a.s.l.)**	**Aspect (1)**	**Slope (2)**	**Landscape surrounding (3)**	**Additional sampling site on the border of vineyard**	**FD infected***	**BN infected***	**Applications of Buprofezin per year**
Chiasso	1023-Pedr	9° 0' 58.46" E	45° 49' 38.25" N	451	NE-NW	>10°	Forest	yes	+	+	0
Mendrisio	1057-Prel	8° 57' 7.07" E	45° 50' 42.97" N	388	NE-NW	<5°	Forest	no			2
Mendrisio	1077-Cort	8° 59' 35.29" E	45° 51' 51.55" N	425	NE-NW	<5°	Open areas	no			2
Vezia	1087-Vezi	8° 55' 56.85" E	46° 1' 17.39" N	334	NE-NW	<5°	Settlements	no	+		2
Rovio	1095-Rovi	8° 58' 39.04" E	45° 56' 3.10" N	424	NE-NW	>10°	Forest	yes		+	0
Mendrisio	1098-Soma	8° 59' 30.34" E	45° 52' 37.53" N	537	NE-NW	>10°	Settlements	no			2
Meride	1118-Meri	8° 57' 23.81" E	45° 53' 26.82" N	589	SE-SW	>10°	Forest	no			0
Castel San Pietro	1123-Gorl	9° 0' 15.27" E	45° 51' 16.73" N	346	SE-SW	<5°	Settlements	no		+	0
Bioggio	1138-Righ	8° 53' 45.11" E	46° 0' 18.88" N	437	SE-SW	>10°	Open areas	no			2
Monteggio	1173-Forn	8° 47' 22.97" E	45° 59' 37.93" N	303	NE-NW	<5°	Forest	no	+		2
Monteggio	1180-Mont	8° 48' 23.78" E	45° 59' 37.67" N	377	SE-SW	>10°	Forest	yes	+	+	2
Croglio	1195-Crog	8° 50' 10.44" E	45° 59' 19.89" N	290	NE-NW	>10°	Open areas	yes	+	+	2
Maggia	169-Magg	8° 42' 32.09" E	46° 14' 57.70" N	375	NE-NW	>10°	Open areas	yes			0
Camorino	213-Camo	8° 00' 51.02" E	46° 09' 53.68" N	407	NE-NW	>10°	Forest	no	+	+	2
Arbedo	225-Arbe	8° 02' 27.97" E	46° 13' 03.05" N	238	NE-NW	<5°	Settlements	yes	+	+	0
Sementina	252-Seme	8° 59' 32.20" E	46° 10' 38.63" N	218	SE-SW	<5°	Open areas	yes			1
Cugnasco-Gerra	312-Cugn	8° 54' 05.64" E	46° 10' 31.74" N	199	SE-SW	<5°	Open areas	no	+	+	2
Monte Carasso	321-Cara	8° 01' 03.69" E	46° 12' 18.86" N	233	SE-SW	<5°	Forest	no			0
Bellinzona	324-Bell	8° 01' 40.72" E	46° 11' 00.56" N	336	NE-NW	>10°	Settlements	no		+	0
Claro	339-Clar	8° 01' 45.31" E	46° 14' 44.17" N	257	NE-NW	<5°	Open areas	no		+	0
Lumino	341-Lumi	8° 03' 40.16" E	46° 13' 52.76" N	298	SE-SW	>10°	Settlements	yes		+	0
Claro	375-Razz	8° 01 52.34" E	46° 15' 04.67" N	412	NE-NW	>10°	Forest	yes			0
Gudo	391-Gudo	8° 55' 48.91" E	46° 10' 26.26" N	210	SE-SW	<5°	Open areas	no		+	0
Camorino	436-Caco	8° 59' 36.42" E	46° 9' 33.87" N	220	NE-NW	<5°	Settlements	no			0
Sementina	454-Mond	8° 58' 28.17" E	46° 10' 52.58" N	372	SE-SW	>10°	Forest	no	+		2
Gordola	456-Gord	8° 51' 54.69" E	46° 10' 57.89" N	319	SE-SW	>10°	Settlements	yes			0
Lavertezzo	458-Lave	8° 53' 16.87" E	46° 10' 47.25" N	336	SE-SW	>10°	Open areas	no			2
Giornico	46-Gior	8° 52' 44.70" E	46° 24' 08.92" N	398	SE-SW	<5°	Forest	no		+	0
Cadenazzo	474-Cade	8° 55' 11.89" E	46° 8' 57.12" N	209	NE-NW	<5°	Open areas	no	+	+	2
Losone	608-Loso	8° 45' 10.05" E	46° 10' 33.32" N	228	NE-NW	<5°	Open areas	no	+	+	0
Giornico	61-Negh	8° 52' 38.46" E	46° 23' 48.12" N	378	NE-NW	<5°	Forest	yes		+	0
Ascona	636-Asco	8° 46' 36.48" E	46° 9' 19.30" N	205	SE-SW	<5°	Open areas	no			2
Monteceneri	669-Biro	8° 55' 36.19" E	46° 7' 29.68" N	512	SE-SW	>10°	Open areas	yes			0
Monteceneri	736-Bica	8° 55' 59.18" E	46° 7' 3.79" N	511	NE-NW	>10°	Settlements	no			0
Malvaglia	73-Malv	8° 59' 02.56" E	46° 24' 34.07" N	451	NE-NW	>10°	Open areas	yes		+	0
Mezzovico-Vira	745-Mzvc	8° 55' 9.31" E	46° 5' 17.81" N	411	SE-SW	<5°	Settlements	no			0
Lamone	760-Lamo	8° 56' 21.96" E	46° 2' 40.70" N	428	NE-NW	>10°	Open areas	yes	+		2
Stabio	778-Stab	8° 55' 38.85" E	45° 51' 19.62" N	421	SE-SW	<5°	Forest	yes	+	+	2
Novazzano	780-Nova	8° 58' 0.02" E	45° 50' 32.65" N	401	NE-NW	>10°	Settlements	no			2
Besazio	796-Besa	8° 57' 1.36" E	45° 52' 32.34" N	554	NE-NW	<5°	Forest	yes		+	2
Balerna	802-Mezz	8° 59' 52.93" E	45° 51' 7.02" N	324	NE-NW	<5°	Settlements	no	+	+	2
Mendrisio	862-Rabe	8° 58' 3.38" E	45° 52' 23.29" N	378	SE-SW	>10°	Open areas	no			2
Ludiano	86-Ludi	8° 58' 11.39" E	46° 24' 57.92" N	459	SE-SW	>10°	Forest	no		+	1
Biasca	8-Bias	8° 57' 47.60" E	46° 21' 54.36" N	309	SE-SW	<5°	Forest	no		+	0
Mendrisio	912-Ranc	8° 58' 18.04" E	45° 52' 18.70" N	343	SE-SW	<5°	Settlements	no	+		2
Bioggio	927-Biog	8° 54' 32.58" E	46° 0' 54.89" N	310	SE-SW	<5°	Settlements	no			2
Collina d'oro	979-Coll	8° 56' 6.77" E	45° 59' 31.07" N	383	SE-SW	>10°	Settlements	no			2
Porza	995-Porz	8° 57' 19.85" E	46° 1' 33.67" N	442	SE-SW	>10°	Settlements	no		+	2

(1) NE-NW: from Northeast to Northwest; SE-SW: from Southeast to Southwest

(2) slope of surfaces in degree

(3) the dominant landscape element (>50% cover) surrounding the vineyard within a radius of 500 m

(*) vineyard with phytoplasma-infected grapevines detected in the last ten years.

Table 6:A2. Phytoplasma isolates used in the present work and GenBank accession numbers.

Isolates from this study	**Host**	**Accession number**			
		16-23 SrRNA	***SecY***	***tuf***	***rplV***
Ho-69	*Hyalesthes obsoletus*	see KR024259	KP635226	KR029136	-
Ho-98	*Hyalesthes obsoletus*	KP941110	KT310178	KR029137	-
Ho-112	*Hyalesthes obsoletus*	see KR024259	KP635232	see KR029140	-
Ho-167	*Hyalesthes obsoletus*	KR024259	KP635233	see KR029136	-
Ho-240	*Hyalesthes obsoletus*	KR024258	KP635227	see KR029136	-
Ho-263	*Hyalesthes obsoletus*	KP941109	KP635235	see KR029140	-
Ho-271	*Hyalesthes obsoletus*	see KR024258	KP635234	see KR029140	-
Ho-357	*Hyalesthes obsoletus*	KR024255	KP635231	KR029140	-
Rc-255	*Reptalus cuspidatus*	KR024256	KP635229	KR029139	-
Rc-264	*Reptalus cuspidatus*	KR024257	KP635230	see KR029139	-
Rp-202	*Reptalus panzeri*	see KR024259	KP635228	KR029138	-
Oi-46	*Orientus ishidae*	see KR024261	KR350640	-	KR350643
Oi-63	*Orientus ishidae*	KR024261	KR350641	-	-
Oi-78	*Orientus ishidae*	KR024260	KR350639	-	KR350644
Oi-368	*Orientus ishidae*	see KP890031	KT371524	-	KT371528
Oi-369	*Orientus ishidae*	see KR024261	KT371525	-	KT371529
Oi-370	*Orientus ishidae*	see KR024261	KT371526	-	KT371530
St-182	*Scaphoideus titanus*	KP890031	KR350642	-	KR350645
St-371	*Scaphoideus titanus*	see KP890031	KT371527	-	KT371531
HYT1	*Hyalesthes obsoletus*	-	-	KC243393	-
HYT2	*Hyalesthes obsoletus*	-	-	KC243394	-
CrHo13_1183	*Hyalesthes obsoletus*	-	-	KJ469707	-
CrHo12_601	*Hyalesthes obsoletus (Vitis vinifera, Con*	-	-	KJ469708	-
CrHo12_650	*Hyalesthes obsoletus (Vitis vinifera, Urti*	-	-	KJ469709	-
HoU93	*Hyalesthes obsoletus*	-	-	KP337325	-
HoU17	*Hyalesthes obsoletus*	-	-	KP337324	-
Ho-TV-16a	*Hyalesthes obsoletus*	-	KT310183	-	-
Ho-TV-16a	*Hyalesthes obsoletus*	-	KT310184	-	-
H160	*Hyalesthes obsoletus*	-	FN813288	-	-
PO	*Hyalesthes obsoletus*	-	AM992082	-	-
Se-At1	*Hyalesthes obsoletus (Vitis vinifera, Urti*	-	KJ469711	-	-
Se-At2	*Hyalesthes obsoletus*	-	KJ469712	-	-
Se-At3	*Hyalesthes obsoletus*	-	KJ469713	-	-
Se-At5	*Hyalesthes obsoletus (Vitis vinifera, Con*	-	KJ469715	-	-
Rpg39	*Reptalus panzeri*	-	KC703037	-	-
Rqg60	*Reptalus quinquecostatus*	-	KC703039	-	-
FD70	*Orientus ishidae*	-	HM367596	-	-
FD-D	*Orientus ishidae*	-	HM367597	-	-
10K	*Scaphoideus titanus*	-	KJ908967	-	-
Se-At4	*Vitis vinifera*	-	KJ469714	-	-

R49/15	*Vitis vinifera*	-	-	FJ394551	-
R47/5	*Vitis vinifera*	-	-	FJ394552	-
BN-Fc6	*Vitis vinifera*	-	-	GU220558	-
BN-Op37	*Vitis vinifera*	-	-	GU220562	-
4MN	*Vitis vinifera*	-	-	FJ441241	-
6MN	*Vitis vinifera*	-	-	FJ441242	-
G1 (?)	*Vitis vinifera*	-	-	KP337326	-
IL11-03	*Vitis vinifera*	-	-	EU717121	-
IL14-3	*Vitis vinifera*	-	-	EU717123	-
DRC3	*Vitis vinifera*	-	-	EU717128	-
Vv-FE-57	*Vitis vinifera*	-	KT310179	-	-
Vv-PG-67	*Vitis vinifera*	-	KT310180	-	-
Vv-AL-228	*Vitis vinifera*	-	KT310181	-	-
Vv-AO-333	*Vitis vinifera*	-	KT310182	-	-
CH1	*Vitis vinifera*	-	AM992089	-	-
SB5	*Vitis vinifera*	-	FN813272	-	-
Aa16	*Vitis vinifera*	-	KJ145389	-	-
Mca28	*Vitis vinifera*	-	KJ145390	-	-
149	*Vitis vinifera*	-	KJ145374	-	-
FD70	*Vitis vinifera*	-	AF458383	-	AY197663
FD-C	*Vitis vinifera*	-	AY197688	-	AY197665
V00-SP5	*Vitis vinifera*	-	AM397288	-	-
FD2000	*Vitis vinifera*	-	AY093581	-	-
VI04-C28	*Vitis vinifera*	-	AM397287	-	FN562167
FD57	*Vitis vinifera*	-	EF581170	-	EF581167
FD68	*Vitis vinifera*	-	EF581169	-	EF581168
V04-11-50	*Vitis vinifera*	-	AM397286	-	-
FD-D	*Vitis vinifera*	-	-	-	AY197664
Vv-AO262	*Vitis vinifera*	-	-	-	FN562168
VI04-D004-03	*Vitis vinifera*	-	-	-	FN562165
VI04-248-04	*Vitis vinifera*	-	-	-	FN562168
V04-11-55	*Vitis vinifera*	-	-	-	FN562166
V04-11-25	*Vitis vinifera*	-	-	-	FN562165
ALY	*Alnus glutinosa*	-	AY197684	-	AY197666
ALY882	*Alnus glutinosa*	-	-	-	AY197662
ALY1068	*Alnus glutinosa*	-	-	-	AY197667
STOL11	*Capsicum annuum*	-	JQ797668	JQ797670	-
CL-AL31	*Clematis vitalba*	-	FJ648480	-	FN562166
CL-TV37	*Clematis vitalba*	-	FJ648472	-	-
CL-UD147	*Clematis vitalba*	-	-	-	FN811139
D_Ca	*Convolvulus arvensis*	-	JQ977710	-	-
STOLC	*Lycopersicum esculentum*	-	KT310185	-	-
U79 (?)	*Urtica dioica*	-	-	KP337327	-
B_Ud	*Urtica dioica*	-	JQ977708	-	-

Table 6:A3 List of Auchenorrhyncha species detected in 2011 in 48 vineyards South of Swiss Alps.

Species	
	Cixiidae
1	Cixius cunicularius
2	Cixius nervosus
3	Hyalesthes obsoletus
4	Reptalus cuspidatus
5	Reptalus panzeri
	Delphacidae
6	Acanthodelphax denticauda
7	Acanthodelphax spinosa
8	Anakelisia perspicillata
9	Asiraca clavicornis
10	Conomelus lorifer
11	Delphacodes venosus
12	Dicranotropis hamata
13	Ditropsis flavipes
14	Ditropis pteridis
15	Falcotoya minuscula
16	Horvathianella palliceps
17	Javesella dubia
18	Kelisia guttulifera
19	Kelisia monoceros
20	Kelisia praecox
21	Laodelphax striatella
22	Megadelphax sordidula
23	Muellerianella extrusa
24	Muellerianella fairmairei
25	Ribautodelphax albostriata
26	Ribautodelphax angulosa
27	Ribautodelphax collina
28	Ribautodelphax imitans
29	Ribautodelphax pungens
30	Ribautodelphax vinealis
31	Stenocranus major
32	Toya propinqua
33	Xanthodelphax straminea
	Issidae
34	Agalmatium flavescens
35	Issus coleoptratus
	Membracidae
36	Centrotus cornutus
37	Gargara genistae
38	Stictocephala bisonia
	Cercopidae
39	Cercopis sanguinolenta
40	Cercopis vulnerata
	Aphrophoridae
41	Aphrophora alni
42	Aphrophora major
43	Lepyronia coleoptrata
44	Neophilaenus campestris
45	Philaenus spumarius
	Dictyopharidae
46	Dictyophara europaea
	Flatidae
47	Metcalfa pruinosa
	Tettigometridae
48	Tettigometra atra
49	Tettigometra virescens
	Ulopidae
50	Utecha trivia
	Cicadellidae
51	Acericerus ribauti
52	Aconurella prolixa
53	Adarrus exornatus
54	Alebra albostriella
55	Allygidius abbreviatus
56	Allygidius atomarius
57	Allygus mixtus
58	Allygus modestus
59	Anaceratagallia ribauti
60	Anoplotettix fuscovenosus
61	Anoscopus albifrons
62	Anoscopus flavostriatus
63	Anoscopus serratulae
64	Aphrodes makarovi
65	Arboridia erecta
66	Arboridia parvula
67	Arboridia ribauti
68	Arboridia spathulata
69	Arocephalus languidus
70	Arocephalus longiceps
71	Arthaldeus pascuellus
72	Arthaldeus striifrons
73	Balclutha punctata
74	Balclutha saltuella
75	Chiasmus conspurcatus
76	Chlorita paolii
77	Chlorita tamaninii
78	Chlorita viridula
79	Cicadula quadrinotata
80	Cicadella viridis
81	Conosanus obsoletus
82	Deltocephalus pulicaris
83	Dikraneura variata
84	Doratura stylata
85	Dryodurgades reticulatus
86	Ebarrius cognatus
87	Elymana sulphurella
88	Emelyanoviana mollicula
89	Empoasca affinis
90	Empoasca decipiens
91	Empoasca pteridis
92	Empoasca vitis
93	Errastunus ocellaris
94	Errhomenus brachypterus
95	Erythria pedemontana
96	Eupelix cuspidata
97	Eupteryx atropunctata
98	Eupteryx aurata
99	Eupteryx calcarata
100	Eupteryx curtisii
101	Eupteryx decemnotata
102	Eupteryx heydenii
103	Eupteryx notata
104	Eupteryx stachydearum
105	Eupteryx urticae
106	Eupteryx vittata
107	Euscelis incisus
108	Eurhadina pulchella
109	Euscelidius variegatus
110	Evacanthus acuminatus
111	Fieberiella florii
112	Forcipata major
113	Goniagnathus brevis
114	Graphocephala fennahi
115	Graphocraerus ventralis
116	Hephathus nanus
117	Japananus hyalinus
118	Jassargus allobrogicus
119	Jassargus bisubulatus
120	Jassargus obtusivalvis
121	Ledra aurita
122	Macrosteles cristatus
123	Macrosteles fieberi
124	Macrosteles frontalis
125	Macropsis fuscula
126	Macrosteles horvathi
127	Macrosteles laevis
128	Macrosteles lividus
129	Macrosteles ossiannilssoni

130	Macrosteles quadripunctulatus
131	Macrosteles sexnotatus
132	Macrosteles viridigriseus
133	Megophthalmus scanicus
134	Mocydia crocea
135	Mocydiopsis monticola
136	Neoaliturus fenestratus
137	Oncopsis sp.
138	Ophiola decumana
139	Orientus ishidae
140	Penthimia nigra
141	Planaphrodes bifasciatus
142	Planaphrodes trifasciatus
143	Psammotettix alienus
144	Psammotettix cephalotes
145	Psammotettix confinis
146	Psammotettix dubius
147	Psammotettix excisus
148	Psammotettix helvolus
149	Recilia coronifera
150	Recilia horvathi
151	Recilia schmidtgeni
152	Rhopalopyx elongatus
153	Rhopalopyx preyssleri
154	Ribautiana debilis
155	Ribautiana tenerrima
156	Scaphoideus titanus
157	Selenocephalus griseus
158	Speudotettix subfusculus
159	Streptanus aemulans
160	Stroggylocephalus agrestis
161	Thamnotettix dilutior
162	Thamnotettix exemptus
163	Turrutus socialis
164	Zonocyba bifasciata
165	Zygina hyperici
166	Zyginidia pullula
167	Zygina rhamni

Figure 6 :A4 Sample-based rarefaction curve for species richness of Auchenorrhyncha fauna recorded in the 48 vineyards sampled in 2011. These curves are based on the output of the R 2.15.1 (Core Team, 2012) function rarc from the package rich.rarc which performs rarefaction using resampling with replacement.

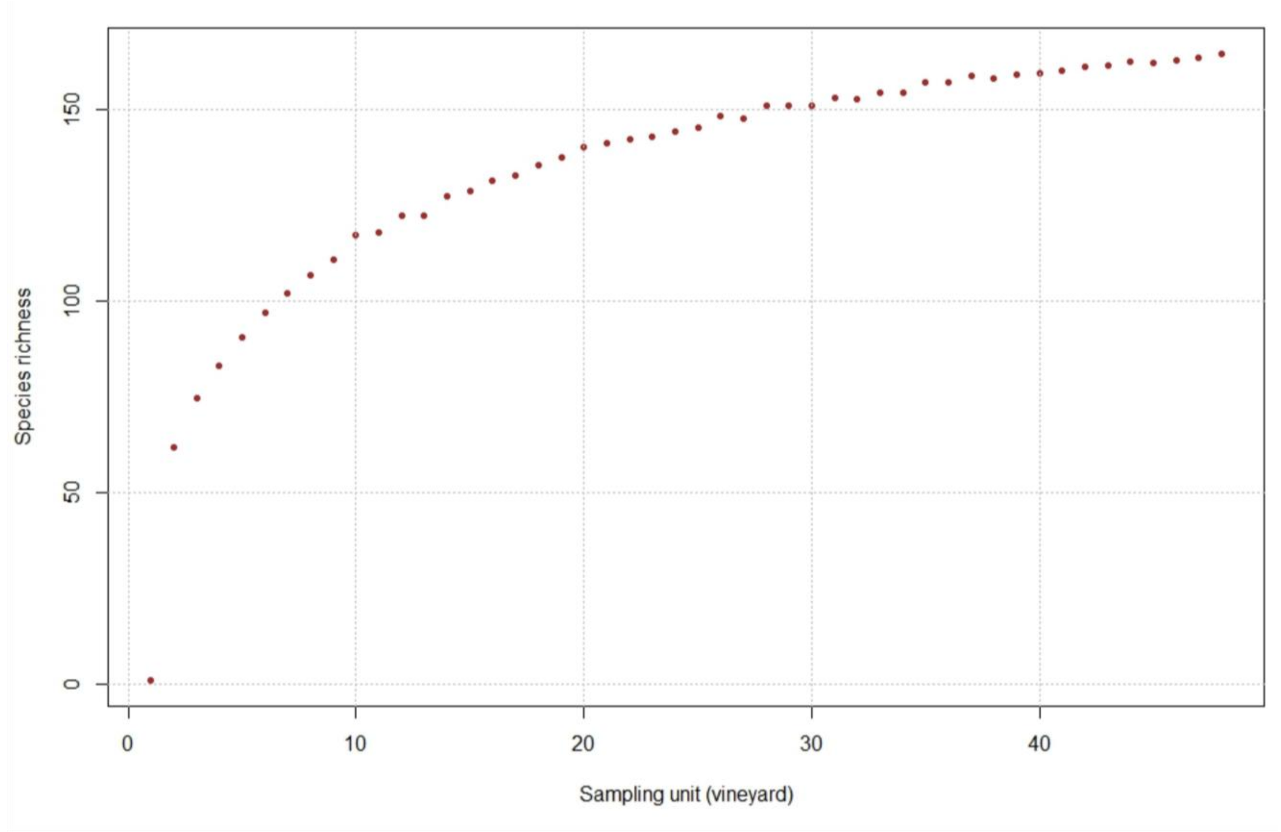

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Table 6:A5 Auchenorrhyncha species known and potential vectors of phytoplasma, selected according to the literature and after field data evaluation. We selected five further species (no code reference) as potential vectors in vineyards where outbreaks of phytoplasma disease were observed and no presence of known vectors was recorded. Batches of specimens of each species were tested by means of molecular analyses.

	Species	**Acronym**	**Ind./Sites**	**Phytoplasma**	**Code Reference**
1	*Allygus mixtus*	A.mixt	3/3	16SrIX	1, 2
2	*Anaceratagallia ribauti*	A.riba	4152/48	16SrXII	3, 4
3	*Anoplotettix fuscovenosus*	A.fusc	34/16	16SrV	5
4	*Aphrodes makarovi*	A.maka	804/37	16SrIII-P 16SrI-B 16SrI-C	6, 7
5	*Aphrophora alni*	A.alni	20/12	16SrI-B	7
6	*Aphrophora major*	A.majo	27/13	16SrI-B	7
7	*Arocephalus longiceps*	A.long	2649/42	-	-
8	*Centrotus cornutus*	C.corn	11/8	-	-
9	*Dictyophara europaea*	D.euro	18/2	16SrV, 16SrXII	8, 9
10	*Euscelis incisus*	E.inci	1381/47	16SrV	10, 5
11	*Fieberella florii*	F.flor	9/7	16Sr III	11, 1, 12
12	*Hyalesthes obsoletus*	H.obso	529/34	16SrXII	13
13	*Issus coleoptratus*	I.cole	185/9	-	-
14	*Japananus hyalinus*	J.hyal	2/1	-	-
15	*Macrosteles cristatus*	M.cris	2856/39	16SrI, 16SrIII	14
16	*Macrosteles viridigriseus*	M.viri	1115/11	16SrI-C	15
17	*Megophthalmus scanicus*	M.scan	262/34	AY	16
18	*Metcalfa pruinosa*	M.prui	18/9	16SrXII	17
19	*Neoaliturus fenestratus*	N.fene	80/20	16SrI,16SrII, 16SrXII	18, 19, 20
20	*Orientus ishidae*	O.ishi	48/14	16SrV	21
21	*Penthimia nigra*	P.nigr	8/5	-	-
22	*Philaenus spumarius*	P.spum	119/35	16SrI-B, 16SrI-C	7
23	*Psammotettix confinis*	P.conf	3493/48	16SrI-B, 16SrI-C	22
24	*Reptalus cuspidatus*	R.cusp	2119/26	16SrXII-A	23
25	*Reptalus panzeri*	R.panz	18/3	16SrXII-A	24
26	*Scaphoideus titanus*	S.tita	2299/35	16SrV	25
27	*Thamnotettix dilutior*	T.dilu	33/6	16SrXII	26

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Table 6:A6 Relationship between the Mean Abundance and occurrence of leafhopper species known and potential vectors of phytoplasma from vineyards in southern Switzerland. Abundance (mean) refers to the total number of adults collected in seven sampling periods by means of four sampling methods. For species acronyms see Table 6:A5.

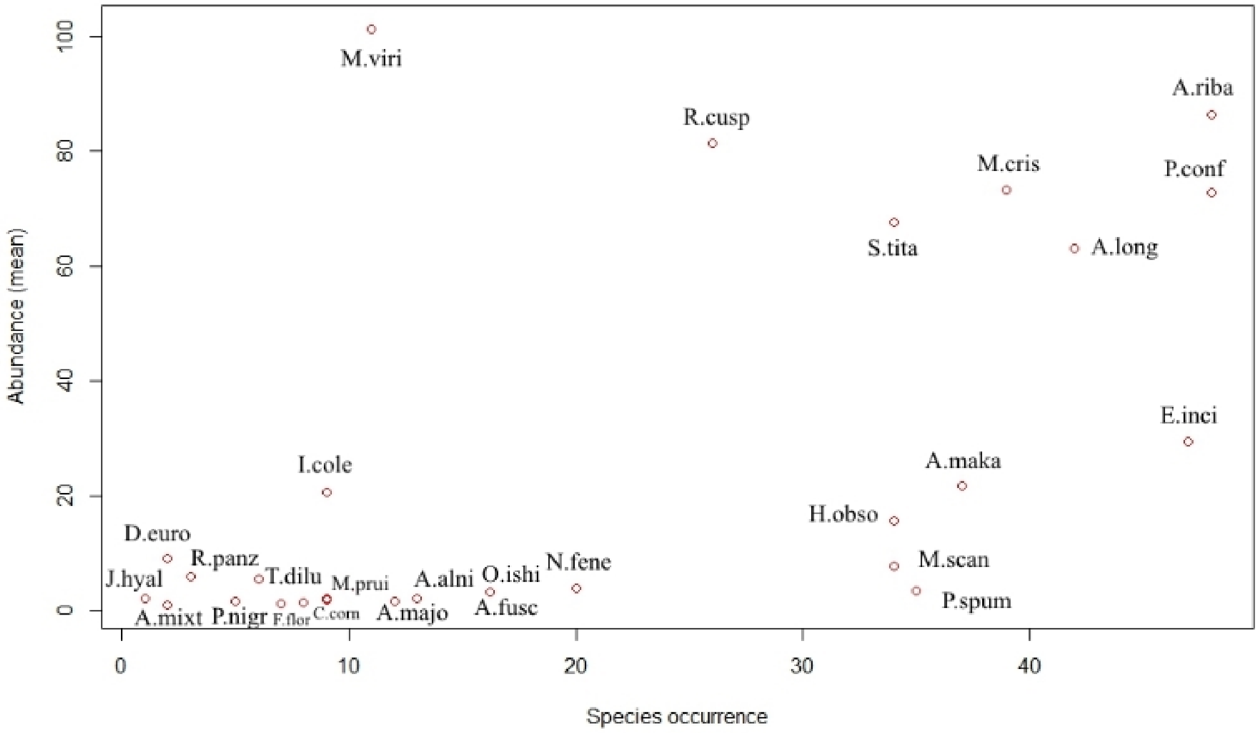


Table 6:A7 List of leafhopper samples considered for molecular analyses and genetic characterization, with some details on insect trapping and trap location.

ID	Vineyard code	Disease detected (2004-2013)	Trap location	Sampling method	Species	No. Ind.
1	86-Ludi	BN	vine	Sticky trap	*Scaphoideus titanus*	3
2	436-Caco		vine	Sticky trap	*Scaphoideus titanus*	6
3	73-Malv	BN	vine	Sticky trap	*Scaphoideus titanus*	10
4	213-Camo	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	1
5	213-Camo	FD-BN	vine	Sticky trap	*Anoplotettix fuscovenosus*	4
6	46-Gior	BN	vine	Sticky trap	*Scaphoideus titanus*	13
7	341-Lumi	BN	vine	Sticky trap	*Scaphoideus titanus*	19
8	8-Bias	BN	vine	Sticky trap	*Scaphoideus titanus*	14
9	61-Negh	BN	vine	Sticky trap	*Scaphoideus titanus*	11
10	341-Lumi	BN	border vineyard	Sticky trap	*Reptalus cuspidatus*	1
11	375-Razz		vine	Sticky trap	*Scaphoideus titanus*	16
12	375-Razz		border vineyard	Sticky trap	*Thamnotettix dilutior*	1
13	736-Bica		vine	Sticky trap	*Anoplotettix fuscovenosus*	1
14	1173-Forn	FD	vine	Sticky trap	*Scaphoideus titanus*	1
15	669-Biro		vine	Sticky trap	*Scaphoideus titanus*	6
16	736-Bica		vine	Sticky trap	*Scaphoideus titanus*	19
17	760-Lamo	FD	vine	Sticky trap	*Anoplotettix fuscovenosus*	1
18	760-Lamo	FD	vine	Sticky trap	*Orientus ishidae*	2
19	745-Mzvc		vine	Sticky trap	*Scaphoideus titanus*	12
20	1195-Crog	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	2
21	1180-Mont	FD-BN	border vineyard	Sticky trap	*Orientus ishidae*	1
22	927-Biog		vine	Sticky trap	*Scaphoideus titanus*	1
23	736-Bica		enbankment	Sticky trap	*Scaphoideus titanus*	2
24	669-Biro		vine	Sticky trap	*Scaphoideus titanus*	4
25	736-Bica		vine	Sticky trap	*Scaphoideus titanus*	4
26	745-Mzvc		vine	Sticky trap	*Scaphoideus titanus*	12
27	1138-Righ		enbankment	Sticky trap	*Orientus ishidae*	1
28	1195-Crog	FD-BN	border vineyard	Sticky trap	*Japananus hyalinus*	1
29	1195-Crog	FD-BN	enbankment	Sticky trap	*Macrosteles cristatus*	1
30	1180-Mont	FD-BN	border vineyard	Sticky trap	*Orientus ishidae*	1
31	1138-Righ		enbankment	Sticky trap	*Scaphoideus titanus*	1
32	73-Malv	BN	vine	Sticky trap	*Scaphoideus titanus*	15
33	46-Gior	BN	vine	Sticky trap	*Scaphoideus titanus*	9
34	46-Gior	BN	vine	Sticky trap	*Scaphoideus titanus*	17
35	61-Negh	BN	border vineyard	Sticky trap	*Scaphoideus titanus*	1
36	213-Camo	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	3
37	436-Caco		vine	Sticky trap	*Scaphoideus titanus*	6
38	8-Bias	BN	vine	Sticky trap	*Scaphoideus titanus*	10
39	61-Negh	BN	border vineyard	Sticky trap	*Orientus ishidae*	1
40	61-Negh	BN	vine	Sticky trap	*Scaphoideus titanus*	8
41	862-Rabe		vine	Sticky trap	*Scaphoideus titanus*	3
42	745-Mzvc		vine	Sticky trap	*Scaphoideus titanus*	9
43	778-Stab	FD-BN	vine	Sticky trap	*Psammotettix confinis*	1
44	1095-Rovi	BN	border vineyard	Sticky trap	*Orientus ishidae*	1
45	862-Rabe		vine	Sticky trap	*Reptalus panzeri*	1
46	778-Stab	FD-BN	border vineyard	Sticky trap	*Orientus ishidae*	3
47	796-Besa	BN	border vineyard	Sticky trap	*Orientus ishidae*	3
48	1095-Rovi	BN	vine	Sticky trap	*Hyalesthes obsoletus*	1
49	778-Stab	FD-BN	vine	Sticky trap	*Neoaliturus fenestratus*	1
50	778-Stab	FD-BN	border vineyard	Sticky trap	*Macrosteles viridigriseus*	1
51	796-Besa	BN	border vineyard	Sticky trap	*Fieberella florii*	1
52	1023-Pedr	FD-BN	enbankment	Sticky trap	*Thamnotettix dilutior*	1
53	1095-Rovi	BN	enbankment	Sticky trap	*Scaphoideus titanus*	1
54	778-Stab	FD-BN	vine	Sticky trap	*Macrosteles viridigriseus*	1
55	1118-Meri		vine	Sticky trap	*Orientus ishidae*	1
56	780-Nova		vine	Sticky trap	*Psammotettix confinis*	2
57	780-Nova		vine	Sticky trap	*Macrosteles viridigriseus*	6
58	912-Ranc	FD	vine	Sticky trap	*Neoaliturus fenestratus*	1
59	796-Besa	BN	border vineyard	Sticky trap	*Orientus ishidae*	9
60	1095-Rovi	BN	border vineyard	Sticky trap	*Orientus ishidae*	13
61	341-Lumi	BN	border vineyard	Sticky trap	*Scaphoideus titanus*	6
62	375-Razz		vine	Sticky trap	*Scaphoideus titanus*	8
63	862-Rabe		vine	Sticky trap	*Orientus ishidae*	1
64	339-Clar	BN	vine	Sticky trap	*Scaphoideus titanus*	12
65	341-Lumi	BN	vine	Sticky trap	*Fieberella florii*	1
66	802-Mezz	FD-BN	vine	Sticky trap	*Neoaliturus fenestratus*	1
67	252-Seme		vine	Sticky trap	*Orientus ishidae*	1
68	252-Seme		vine	Sticky trap	*Scaphoideus titanus*	4
69	1195-Crog	FD-BN	vine	Sticky trap	*Hyalesthes obsoletus*	3
70	321-Cara		vine	Sticky trap	*Scaphoideus titanus*	3
71	745-Mzvc		vine	Sticky trap	*Scaphoideus titanus*	2
72	1195-Crog	FD-BN	border vineyard	Sticky trap	*Japananus hyalinus*	2
73	608-Loso	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	7
74	1087-Vezi	FD	vine	Sticky trap	*Fieberella florii*	1
75	736-Bica		vine	Sticky trap	*Scaphoideus titanus*	1
76	391-Gudo	BN	vine	Sticky trap	*Scaphoideus titanus*	7
77	225-Arbe	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	2
78	760-Lamo	FD	border vineyard	Sticky trap	*Orientus ishidae*	1
79	736-Bica		enbankment	Sticky trap	*Anoplotettix fuscovenosus*	1
80	456-Gord		enbankment	Sticky trap	*Scaphoideus titanus*	3
81	341-Lumi	BN	vine	Sticky trap	*Scaphoideus titanus*	12
82	213-Camo	FD-BN	vine	Sticky trap	*Thamnotettix dilutior*	1
83	436-Caco		vine	Sticky trap	*Orientus ishidae*	1
84	213-Camo	FD-BN	vine	Sticky trap	*Anoplotettix fuscovenosus*	2
85	61-Negh	BN	vine	Sticky trap	*Anoplotettix fuscovenosus*	1
86	341-Lumi	BN	vine	Sticky trap	*Reptalus panzeri*	2
87	324-Bell	BN	vine	Sticky trap	*Scaphoideus titanus*	1
88	61-Negh	BN	vine	Sticky trap	*Hyalesthes obsoletus*	1
89	73-Malv	BN	vine	Sticky trap	*Scaphoideus titanus*	2
90	375-Razz		vine	Sticky trap	*Thamnotettix dilutior*	1
91	8-Bias	BN	vine	Sticky trap	*Scaphoideus titanus*	1
92	341-Lumi	BN	border vineyard	Sticky trap	*Hyalesthes obsoletus*	6
93	1195-Crog	FD-BN	enbankment+vine	Sticky trap	*Hyalesthes obsoletus*	21
94	321-Cara		vine	Sticky trap	*Scaphoideus titanus*	8
95	252-Seme		vine	Sticky trap	*Reptalus cuspidatus*	1
96	862-Rabe		vine	Sticky trap	*Anoplotettix fuscovenosus*	4
97	169-Magg		border vineyard	Sticky trap	*Fieberella florii*	2
98	1095-Rovi	BN	enbankment	Sticky trap	*Hyalesthes obsoletus*	1
99	454-Mond	FD	vine	Sticky trap	*Thamnotettix dilutior*	1
100	1095-Rovi	BN	border vineyard	Sticky trap	*Anoplotettix fuscovenosus*	1
101	608-Loso	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	7
102	169-Magg		border vineyard	Sticky trap	*Anoplotettix fuscovenosus*	1
103	760-Lamo	FD	border vineyard	Sticky trap	*Thamnotettix dilutior*	1
104	760-Lamo	FD	vine	Sticky trap	*Penthimia nigra*	1
105	391-Gudo	BN	vine	Sticky trap	*Scaphoideus titanus*	15
106	456-Gord		border vineyard	Sticky trap	*Thamnotettix dilutior*	2
107	169-Magg		vine	Sticky trap	*Scaphoideus titanus*	9
108	1195-Crog	FD-BN	border vineyard	Sticky trap	*Hyalesthes obsoletus*	1
109	736-Bica		vine	Sticky trap	*Thamnotettix dilutior*	1
110	736-Bica		vine	Sticky trap	*Anoplotettix fuscovenosus*	2
111	760-Lamo	FD	border vineyard	Sticky trap	*Anoplotettix fuscovenosus*	1
112	862-Rabe		vine	Sticky trap	*Hyalesthes obsoletus*	2
113	1195-Crog	FD-BN	vine	Sticky trap	*Anoplotettix fuscovenosus*	1
114	225-Arbe	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	2
115	636-Asco		vine	Sticky trap	*Scaphoideus titanus*	3
116	321-Cara		vine	Sticky trap	*Reptalus cuspidatus*	1
117	321-Cara		vine	Sticky trap	*Neoaliturus fenestratus*	1
118	979-Coll		vine	Sticky trap	*Neoaliturus fenestratus*	1
119	252-Seme		vine	Sticky trap	*Orientus ishidae*	1
120	252-Seme		border vineyard	Sticky trap	*Hyalesthes obsoletus*	1
121	1087-Vezi	FD	vine	Beating tray	*Anoplotettix fuscovenosus*	1
122	1173-Forn	FD	vine	Beating tray	*Thamnotettix dilutior*	1
123	1173-Forn	FD	vine	Beating tray	*Megophthalmus scanicus*	3
124	760-Lamo	FD	vine	Beating tray	*Megophthalmus scanicus*	1
125	912-Ranc	FD	vine	Beating tray	*Megophthalmus scanicus*	1
126	1173-Forn	FD	vine	Beating tray	*Scaphoideus titanus*	3
127	912-Ranc	FD	vine	Beating tray	*Scaphoideus titanus*	2
128	1087-Vezi	FD	vine	Beating tray	*Aphrophora major*	1
129	454-Mond	FD	vine	Beating tray	*Hyalesthes obsoletus*	1
130	608-Loso	FD-BN	vine	Beating tray	*Aphrophora major*	1
131	454-Mond	FD	vine	Beating tray	*Allygus mixtus*	1
132	1195-Crog	FD-BN	vine	Beating tray	*Scaphoideus titanus*	1
133	1087-Vezi	FD	vine	Beating tray	*Metcalfa pruinosa*	1
134	454-Mond	FD	vine	Beating tray	*Scaphoideus titanus*	1
135	608-Loso	FD-BN	vine	Beating tray	*Scaphoideus titanus*	4
136	1023-Pedr	FD-BN	vine	Beating tray	*Issus coleoptratus*	73
137	1180-Mont	FD-BN	vine	Beating tray	*Scaphoideus titanus*	1
138	1173-Forn	FD	vine	Beating tray	*Scaphoideus titanus*	5
139	213-Camo	FD-BN	vine	Beating tray	*Scaphoideus titanus*	14
140	474-Cade	FD-BN	vine	Beating tray	*Metcalfa pruinosa*	1
141	1195-Crog	FD-BN	vine	Beating tray	*Aphrophora major*	1
142	1023-Pedr	FD-BN	vine	Beating tray	*Metcalfa pruinosa*	1
143	213-Camo	FD-BN	vine	Beating tray	*Anoplotettix fuscovenosus*	1
144	1195-Crog	FD-BN	vine	Beating tray	*Aphrophora alni*	1
145	474-Cade	FD-BN	vine	Beating tray	*Aphrophora major*	1
146	225-Arbe	FD-BN	vine	Beating tray	*Scaphoideus titanus*	30
147	225-Arbe	FD-BN	vine	Beating tray	*Scaphoideus titanus*	11
148	213-Camo	FD-BN	vine	Beating tray	*Aphrophora major*	1
149	1195-Crog	FD-BN	vine	Beating tray	*Reptalus cuspidatus*	1
150	1195-Crog	FD-BN	vine	Beating tray	*Issus coleoptratus*	2
151	312-Cugn	FD-BN	vine	Beating tray	*Metcalfa pruinosa*	1
152	1023-Pedr	FD-BN	vine	Beating tray	*Orientus ishidae*	1
153	213-Camo	FD-BN	vine	Beating tray	*Anoplotettix fuscovenosus*	2
154	1180-Mont	FD-BN	vine	Beating tray	*Aphrodes makarovi*	1
155	608-Loso	FD-BN	vine	Beating tray	*Scaphoideus titanus*	3
156	608-Loso	FD-BN	vine	Beating tray	*Allygus mixtus*	1
157	1180-Mont	FD-BN	vine	Beating tray	*Metcalfa pruinosa*	1
158	312-Cugn	FD-BN	vine	Beating tray	*Aphrophora major*	1
159	225-Arbe	FD-BN	vine	Beating tray	*Scaphoideus titanus*	30
160	213-Camo	FD-BN	vine	Beating tray	*Scaphoideus titanus*	6
161	1023-Pedr	FD-BN	vine	Beating tray	*Issus coleoptratus*	16
162	778-Stab	FD-BN	vine	Beating tray	*Centrotus cornutus*	1
163	1180-Mont	FD-BN	vine	Beating tray	*Reptalus cuspidatus*	3
164	1023-Pedr	FD-BN	vine	Beating tray	*Reptalus cuspidatus*	1
165	1180-Mont	FD-BN	vine	Beating tray	*Hyalesthes obsoletus*	3
166	1180-Mont	FD-BN	vine	Beating tray	*Aphrophora major*	1
167	1195-Crog	FD-BN	vine	Beating tray	*Hyalesthes obsoletus*	17
168	312-Cugn	FD-BN	vine	Beating tray	*Philaenus spumarius*	5
169	669-Biro		vine	Beating tray	*Scaphoideus titanus*	20
170	669-Biro		vine	Beating tray	*Scaphoideus titanus*	9
171	1118-Meri		vine	Beating tray	*Scaphoideus titanus*	3
172	1098-Soma		vine	Beating tray	*Neoaliturus fenestratus*	1
173	1098-Soma		vine	Beating tray	*Orientus ishidae*	2
174	375-Razz		vine	Beating tray	*Scaphoideus titanus*	22
175	669-Biro		vine	Beating tray	*Scaphoideus titanus*	25
176	252-Seme		vine	Beating tray	*Scaphoideus titanus*	8
177	1077-Cort		vine	Beating tray	*Neoaliturus fenestratus*	1
178	169-Magg		vine	Beating tray	*Scaphoideus titanus*	21
179	669-Biro		vine	Beating tray	*Scaphoideus titanus*	26
180	1098-Soma		vine	Beating tray	*Scaphoideus titanus*	1
181	169-Magg		vine	Beating tray	*Scaphoideus titanus*	17
182	436-Caco		vine	Beating tray	*Scaphoideus titanus*	13
183	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	20
184	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	22
185	1123-Gorl	BN	vine	Beating tray	*Macrosteles cristatus*	1
186	324-Bell	BN	vine	Beating tray	*Scaphoideus titanus*	16
187	1095-Rovi	BN	vine	Beating tray	*Hyalesthes obsoletus*	1
188	46-Gior	BN	vine	Beating tray	*Aphrophora major*	1
189	46-Gior	BN	vine	Beating tray	*Scaphoideus titanus*	22
190	324-Bell	BN	vine	Beating tray	*Allygus mixtus*	1
191	1095-Rovi	BN	vine	Beating tray	*Scaphoideus titanus*	21
192	86-Ludi	BN	vine	Beating tray	*Aphrodes makarovi*	3
193	391-Gudo	BN	vine	Beating tray	*Scaphoideus titanus*	24
194	46-Gior	BN	vine	Beating tray	*Scaphoideus titanus*	28
195	324-Bell	BN	vine	Beating tray	*Aphrophora alni*	2
196	1095-Rovi	BN	vine	Beating tray	*Psammotettix confinis*	1
197	324-Bell	BN	vine	Beating tray	*Anoplotettix fuscovenosus*	5
198	341-Lumi	BN	vine	Beating tray	*Scaphoideus titanus*	6
199	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	22
200	341-Lumi	BN	vine	Beating tray	*Scaphoideus titanus*	21
201	324-Bell	BN	vine	Beating tray	*Scaphoideus titanus*	19
202	796-Besa	BN	vine	Beating tray	*Reptalus panzeri*	1
203	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	23
204	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	22
205	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	20
206	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	20
207	86-Ludi	BN	vine	Beating tray	*Scaphoideus titanus*	8
208	86-Ludi	BN	vine	Beating tray	*Thamnotettix dilutior*	1
209	995-Porz	BN	vine	Beating tray	*Metcalfa pruinosa*	1
210	391-Gudo	BN	vine	Beating tray	*Scaphoideus titanus*	15
211	61-Negh	BN	vine	Beating tray	*Scaphoideus titanus*	14
212	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	20
213	86-Ludi	BN	vine	Beating tray	*Aphrophora major*	1
214	86-Ludi	BN	vine	Beating tray	*Scaphoideus titanus*	19
215	341-Lumi	BN	vine	Beating tray	*Aphrophora alni*	1
216	341-Lumi	BN	vine	Beating tray	*Aphrophora major*	1
217	8-Bias	BN	vine	Beating tray	*Scaphoideus titanus*	19
218	339-Clar	BN	vine	Beating tray	*Scaphoideus titanus*	24
219	86-Ludi	BN	vine	Beating tray	*Scaphoideus titanus*	21
220	73-Malv	BN	vine	Beating tray	*Scaphoideus titanus*	22
221	73-Malv	BN	vine	Beating tray	*Scaphoideus titanus*	25
222	73-Malv	BN	vine	Beating tray	*Scaphoideus titanus*	16
223	61-Negh	BN	vine	Beating tray	*Aphrophora major*	1
224	86-Ludi	BN	vine	Beating tray	*Arocephalus longiceps*	3
225	61-Negh	BN	vine	Beating tray	*Aphrophora alni*	1
226	391-Gudo	BN	vine	Beating tray	*Hyalesthes obsoletus*	5
227	86-Ludi	BN	vine	Beating tray	*Anoplotettix fuscovenosus*	1
228	61-Negh	BN	vine	Beating tray	*Scaphoideus titanus*	5
229	1095-Rovi	BN	vine	Beating tray	*Scaphoideus titanus*	21
230	341-Lumi	BN	vine	Beating tray	*Issus coleoptratus*	4
231	61-Negh	BN	vine	Beating tray	*Anoplotettix fuscovenosus*	1
232	341-Lumi	BN	vine	Beating tray	*Reptalus panzeri*	3
233	73-Malv	BN	vine	Beating tray	*Aphrodes makarovi*	8
234	341-Lumi	BN	vine	Beating tray	*Reptalus cuspidatus*	1
235	391-Gudo	BN	vine	Beating tray	*Metcalfa pruinosa*	1
236	8-Bias	BN	vine	Beating tray	*Aphrodes makarovi*	1
237	73-Malv	BN	vine	Beating tray	*Reptalus cuspidatus*	1
238	73-Malv	BN	vine	Beating tray	*Scaphoideus titanus*	29
239	341-Lumi	BN	vine	Beating tray	*Scaphoideus titanus*	20
240	995-Porz	BN	vine	Beating tray	*Hyalesthes obsoletus*	1
241	339-Clar	BN	vine	Beating tray	*Megophthalmus scanicus*	1
242	341-Lumi	BN	vine	Beating tray	*Aphrophora alni*	1
243	341-Lumi	BN	vine	Beating tray	*Aphrophora major*	1
244	86-Ludi	BN	vine	Beating tray	*Anoplotettix fuscovenosus*	1
245	73-Malv	BN	vine	Beating tray	*Euscelis incisus*	1
246	341-Lumi	BN	vine	Beating tray	*Scaphoideus titanus*	13
247	1095-Rovi	BN	interrow	D-vac	*Dictyophara europaea*	16
248	1095-Rovi	BN	interrow	D-vac	*Hyalesthes obsoletus*	21
249	1095-Rovi	BN	interrow	D-vac	*Reptalus cuspidatus*	19
250	1095-Rovi	BN	interrow	D-vac	*Reptalus cuspidatus*	20
251	1087-Vezi	FD	interrow	D-vac	*Hyalesthes obsoletus*	1
252	8-Bias	BN	interrow	D-vac	*Hyalesthes obsoletus*	3
253	1095-Rovi	BN	interrow	D-vac	*Hyalesthes obsoletus*	20
254	1095-Rovi	BN	interrow	D-vac	*Hyalesthes obsoletus*	27
255	1095-Rovi	BN	interrow	D-vac	*Reptalus cuspidatus*	17
256	8-Bias	BN	interrow	D-vac	*Reptalus cuspidatus*	2
257	1095-Rovi	BN	interrow	D-vac	*Hyalesthes obsoletus*	20
258	8-Bias	BN	interrow	D-vac	*Scaphoideus titanus*	15
259	8-Bias	BN	interrow	D-vac	*Hyalesthes obsoletus*	8
260	8-Bias	BN	interrow	D-vac	*Reptalus cuspidatus*	12
261	1195-Crog	FD-BN	enbankment	D-vac	*Reptalus cuspidatus*	20
262	1195-Crog	FD-BN	enbankment	D-vac	*Reptalus cuspidatus*	21
263	1195-Crog	FD-BN	enbankment	D-vac	*Hyalesthes obsoletus*	20
264	1195-Crog	FD-BN	enbankment	D-vac	*Reptalus cuspidatus*	20
265	1123-Gorl	BN	interrow	D-vac	*Hyalesthes obsoletus*	2
266	8-Bias	BN	interrow	D-vac	*Scaphoideus titanus*	15
267	1195-Crog	FD-BN	enbankment	D-vac	*Hyalesthes obsoletus*	6
268	1123-Gorl	BN	interrow	D-vac	*Macrosteles cristatus*	20
269	1195-Crog	FD-BN	interrow	D-vac	*Hyalesthes obsoletus*	20
270	1195-Crog	FD-BN	enbankment	D-vac	*Hyalesthes obsoletus*	20
271	1195-Crog	FD-BN	interrow	D-vac	*Hyalesthes obsoletus*	20
272	1195-Crog	FD-BN	enbankment	D-vac	*Reptalus cuspidatus*	20
273	862-Rabe		vine	Beating tray	*Reptalus panzeri*	6
274	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	21
275	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	23
276	736-Bica		vine	Beating tray	*Scaphoideus titanus*	6
277	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	14
278	321-Cara		vine	Beating tray	*Scaphoideus titanus*	15
279	979-Coll		vine	Beating tray	*Scaphoideus titanus*	1
280	169-Magg		vine	Beating tray	*Scaphoideus titanus*	24
281	736-Bica		vine	Beating tray	*Scaphoideus titanus*	20
282	456-Gord		vine	Beating tray	*Scaphoideus titanus*	1
283	736-Bica		vine	Beating tray	*Scaphoideus titanus*	21
284	862-Rabe		vine	Beating tray	*Scaphoideus titanus*	10
285	736-Bica		vine	Beating tray	*Scaphoideus titanus*	31
286	736-Bica		vine	Beating tray	*Scaphoideus titanus*	20
287	736-Bica		vine	Beating tray	*Scaphoideus titanus*	20
288	636-Asco		vine	Beating tray	*Scaphoideus titanus*	6
289	321-Cara		vine	Beating tray	*Hyalesthes obsoletus*	1
290	321-Cara		vine	Beating tray	*Reptalus cuspidatus*	1
291	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	20
292	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	17
293	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	20
294	669-Biro		vine	Beating tray	*Orientus ishidae*	1
295	927-Biog		vine	Beating tray	*Scaphoideus titanus*	3
296	456-Gord		vine	Beating tray	*Scaphoideus titanus*	2
297	669-Biro		vine	Beating tray	*Scaphoideus titanus*	25
298	321-Cara		vine	Beating tray	*Scaphoideus titanus*	7
299	375-Razz		vine	Beating tray	*Scaphoideus titanus*	38
300	169-Magg		vine	Beating tray	*Hyalesthes obsoletus*	2
301	375-Razz		vine	Beating tray	*Reptalus cuspidatus*	2
302	736-Bica		vine	Beating tray	*Scaphoideus titanus*	32
303	862-Rabe		vine	Beating tray	*Hyalesthes obsoletus*	4
304	862-Rabe		vine	Beating tray	*Reptalus panzeri*	4
305	979-Coll		vine	Beating tray	*Scaphoideus titanus*	1
306	375-Razz		vine	Beating tray	*Scaphoideus titanus*	20
307	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	22
308	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	20
309	436-Caco		vine	Beating tray	*Scaphoideus titanus*	6
310	736-Bica		vine	Beating tray	*Scaphoideus titanus*	20
311	745-Mzvc		vine	Beating tray	*Scaphoideus titanus*	22
312	436-Caco		vine	Beating tray	*Metcalfa pruinosa*	1
313	862-Rabe		vine	Beating tray	*Reptalus panzeri*	4
314	636-Asco		vine	Beating tray	*Scaphoideus titanus*	5
315	669-Biro		vine	Beating tray	*Reptalus cuspidatus*	1
316	252-Seme		vine	Beating tray	*Scaphoideus titanus*	4
317	252-Seme		vine	Beating tray	*Metcalfa pruinosa*	2
318	912-Ranc	FD	interrow	D-vac	*Macrosteles cristatus*	30
319	912-Ranc	FD	interrow	D-vac	*Euscelis incisus*	20
320	912-Ranc	FD	interrow	D-vac	*Macrosteles cristatus*	33
321	912-Ranc	FD	interrow	D-vac	*Euscelis incisus*	14
322	912-Ranc	FD	interrow	D-vac	*Psammotettix confinis*	19
323	1180-Mont	FD-BN	interrow	D-vac	*Hyalesthes obsoletus*	17
324	1180-Mont	FD-BN	interrow	D-vac	*Reptalus cuspidatus*	20
325	1180-Mont	FD-BN	interrow	D-vac	*Reptalus cuspidatus*	20
326	1180-Mont	FD-BN	enbankment	D-vac	*Hyalesthes obsoletus*	12
327	1023-Pedr	FD-BN	interrow	D-vac	*Hyalesthes obsoletus*	1
328	1023-Pedr	FD-BN	enbankment	D-vac	*Reptalus cuspidatus*	14
329	213-Camo	FD-BN	enbankment	D-vac	*Reptalus cuspidatus*	20
330	213-Camo	FD-BN	enbankment	D-vac	*Anaceratagallia ribauti*	30
331	213-Camo	FD-BN	enbankment	D-vac	*Reptalus cuspidatus*	27
332	213-Camo	FD-BN	enbankment	D-vac	*Aphrodes makarovi*	15
333	760-Lamo	FD	enbankment	D-vac	*Hyalesthes obsoletus*	18
334	391-Gudo	BN	interrow	D-vac	*Scaphoideus titanus*	3
335	391-Gudo	BN	interrow	D-vac	*Anaceratagallia ribauti*	20
336	312-Cugn	FD-BN	interrow	D-vac	*Anaceratagallia ribauti*	11
337	1195-Crog	FD-BN	enbankment	D-vac	*Hyalesthes obsoletus*	2
338	339-Clar	BN	interrow	D-vac	*Psammotettix confinis*	30
339	339-Clar	BN	interrow	D-vac	*Psammotettix confinis*	30
340	339-Clar	BN	interrow	D-vac	*Anaceratagallia ribauti*	23
341	339-Clar	BN	interrow	D-vac	*Macrosteles cristatus*	20
342	339-Clar	BN	interrow	D-vac	*Scaphoideus titanus*	27
343	339-Clar	BN	interrow	D-vac	*Scaphoideus titanus*	14
344	339-Clar	BN	interrow	D-vac	*Scaphoideus titanus*	20
345	339-Clar	BN	interrow	D-vac	*Scaphoideus titanus*	20
346	339-Clar	BN	interrow	D-vac	*Scaphoideus titanus*	25
347	778-Stab	FD-BN	interrow	D-vac	*Macrosteles cristatus*	20
348	778-Stab	FD-BN	interrow	D-vac	*Macrosteles cristatus*	20
349	778-Stab	FD-BN	interrow	D-vac	*Psammotettix confinis*	32
350	778-Stab	FD-BN	interrow	D-vac	*Macrosteles cristatus*	30
351	778-Stab	FD-BN	interrow	D-vac	*Psammotettix confinis*	20
352	778-Stab	FD-BN	interrow	D-vac	*Psammotettix confinis*	20
353	778-Stab	FD-BN	interrow	D-vac	*Macrosteles cristatus*	20
354	778-Stab	FD-BN	interrow	D-vac	*Anaceratagallia ribauti*	12
355	778-Stab	FD-BN	interrow	D-vac	*Hyalesthes obsoletus*	5
356	436-Caco		interrow	D-vac	*Reptalus cuspidatus*	22
357	436-Caco		interrow	D-vac	*Hyalesthes obsoletus*	12
358	436-Caco		interrow	D-vac	*Hyalesthes obsoletus*	20
359	778-Stab	FD-BN	interrow	D-vac	*Psammotettix confinis*	25
360	778-Stab	FD-BN	interrow	D-vac	*Macrosteles cristatus*	25
361	778-Stab	FD-BN	interrow	D-vac	*Psammotettix confinis*	25
362	778-Stab	FD-BN	interrow	D-vac	*Macrosteles cristatus*	25
363	778-Stab	FD-BN	interrow	D-vac	*Psammotettix confinis*	25
364	778-Stab	FD-BN	interrow	D-vac	*Macrosteles cristatus*	25
365	778-Stab	FD-BN	interrow	D-vac	*Anaceratagallia ribauti*	8
366	73-Malv	BN	interrow	D-vac	*Reptalus cuspidatus*	25
367	995-Porz	BN	interrow	D-vac	*Reptalus cuspidatus*	25
368	778-Stab	FD-BN	border vineyard	Sticky trap	*Orientus ishidae*	1
369	778-Stab	FD-BN	vine	Sticky trap	*Orientus ishidae*	1
370	778-Stab	FD-BN	vine	Sticky trap	*Orientus ishidae*	1
371	778-Stab	FD-BN	vine	Sticky trap	*Scaphoideus titanus*	1
					Total	499

Figure 6 :A8 Phylogenetic tree based on 769 bp of the *rplV-rpsC* sequences obtained from *Scaphoideus titanus* and *Orientus ishidae* samples. Swiss samples processed in the current study are evidenced in bold. Bar length is proportional to the number of base substitutions per site. Numbers on the branches are confidence values obtained for 1 000 replicates. GenBank accession numbers and details of the reference phytoplasma strains are listed in Appendix 6:A2. Host: Ag *Alnus glutinosa*; Cv *Clematis vitalba*; Oi *Orientus ishidae*; St *Scaphoideus titanus*; Vv *Vitis vinifera*.

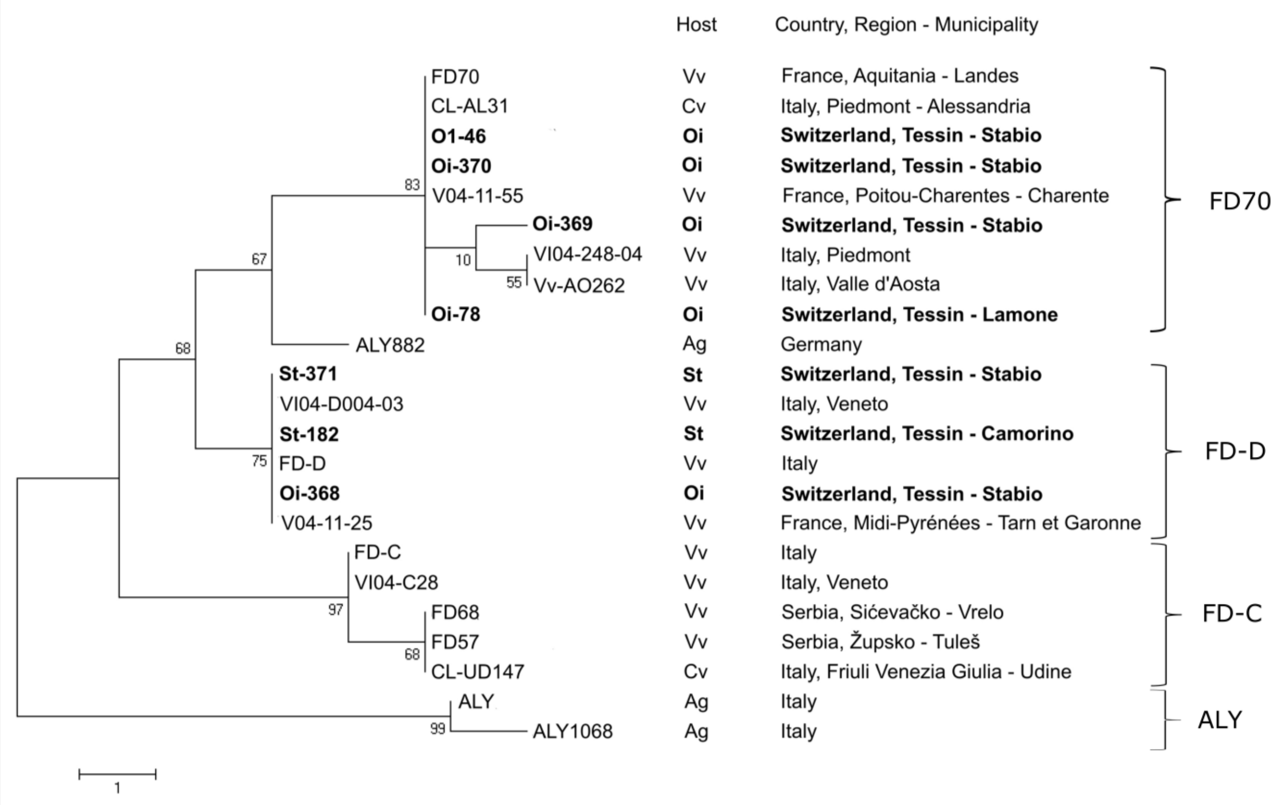
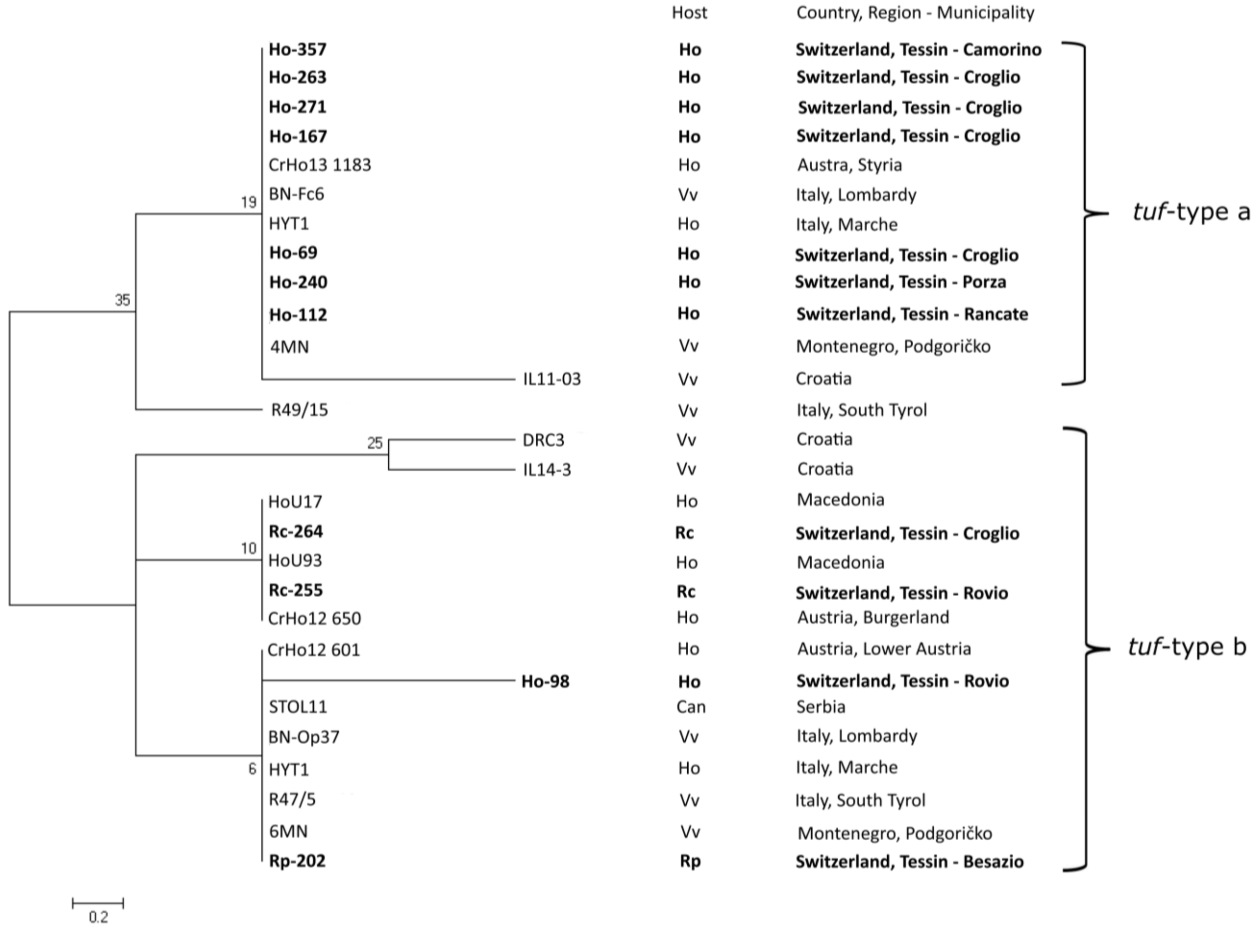


Figure 6 :A9. Phylogenetic tree based on 826 bp of the *tuf* gene sequences obtained from *Hyalesthes obsoletus*, *Reptalus cuspidatus* and *Reptalus panzeri* samples. Swiss samples processed in the current study are evidenced in bold. Bar length is proportional to the number of base substitutions per site. Numbers on the branches are confidence values obtained for 1 000 replicates. GenBank accession numbers and details of the reference phytoplasma strains are listed in Appendix 6:A2. Host: Can *Capsicum annuum*; Ho *Hyalesthes obsoletus*; Rc *Reptalus cuspidatus*; Rp *Reptalus panzeri*; Vv *Vitis vinifera*.



Chapter 7 General discussion

Achieved results and future development

Agriculture is still one of the dominant land uses in Europe. Around half of Europe’s wildlife is associated in one way or another with farmland. This is because traditional land use systems have contributed to create diverse agro-ecosystems which result in the wide range of characteristic and contrasting agricultural landscapes (Orbicon *et al*. 2009). Biodiversity is an important natural property for maintaining stable and productive all kinds of ecosystems, including ago-ecosystems (Cardinale et al. 2006; Hooper et al. 2012). The agriculture sector, therefore, has a shared responsibility in contributing to biodiversity preservation through the application of various sustainable management systems. There are various alternative practices in favor of biodiversity ranging from conservation measures and site-specific crop management, to agricultural activities that are less directly focused on biodiversity conservation.

The concept of sustainable production is a promising approach which requires political and social consensus. Eco-compatible production systems should therefore be conceived to protect the interests of all parties involved and the strategies have to be developed through concerted actions. Approaches to biodiversity conservation also need to move beyond the wild biodiversity focus of strictly protected areas, and to take steps towards an integrated approach based on landscape mosaics where areas in natural/native habitat and areas under eco-agricultural production are interconnected (Scherr and McNeely, 2008).

The present PhD thesis is guided by the above-mentioned principles. Starting with scientific evidences on natural and human stressors affecting ecological communities in the vineyard agroecosystem, it goes on considering multiple biodiversity aspects associated to fields as important components in the agroecosystems, and it ended up highlighting a topical issue of phytosanitary concern which can cause serious threats to both biodiversity and human well-being.

Stressors affecting the plant and leafhopper communities in vineyard agroecosystems

In a pilot study, we sampled Auchenorrhyncha (hereafter leafhoppers) as a model taxon in vineyards in southern Switzerland. Environmental and management variables accounted for most of the variance in the leafhopper assemblage. With increasing management pressure (i.e. pesticide and mowing), the number of indicator species and particularly the specialists (i.e. stenotopic and oligotopic species) decreases dramatically. To promote taxonomic and functional complexity of communities in vineyard systems, we suggest low management pressure with moderate use of pesticide and a low intensity regime of mowing of the embankments. In an extensive study, we investigated abiotic and biotic stressors shaping community assemblages of two trophic levels (plants as producers and leafhoppers as phytophagous). Plant and leafhopper assemblages were mainly affected by topographic variables and biotic interactions across trophic levels. Abiotic filtering processes were relatively more important than biotic ones (plants: 9.6% vs 4.9%; leafhoppers: 14.8% vs 3.8%). However, the total variation in plant and leafhopper communities was explained more by the overlap (12.5% and 20.5%, respectively) between abiotic and biotic variables, suggesting that the biotic relationships within communities are structured by abiotic factors (such as: differentiation of microhabitats at the local level and presence of meadows and grassland in the surroundings). Pairwise co-occurrence analyses highlighted that plant assemblages had a segregated pattern, reasonably caused by the net effect of environmental filtering, heterogeneous resource availability and competitive interactions. On the contrary, most leafhoppers species pairs moved towards an aggregated pattern, probably caused by host feeding differentiation at the local level, different feeding microhabitats on host plants and similar environmental requirements. Our study revealed a specific role of abiotic factors in shaping communities in vineyards in southern Switzerland. Moreover, we showed that a low management pressure and in-field diversification of structures inside vineyards (e.g. embankments) promotes high biological diversity and co-existence of species. Although the main aim of this study was to define the factors shaping plant and leafhopper communities using observational datasets, some important questions remain open, such as: verifying if abiotic factors mediate the effects of competitive species assembly, and clarifying the relationship between communities structure and ecosystem processes and services. In our study significant species segregation across sampling sites could be due to competitive, environmental filtering and dispersal limitation processes acting jointly. Hence, to discern their contribution it would be desirable to conduct experimental manipulations to test specific species assembly rules. An experiment within functional groups could be carried out to determine if segregation patterns revealed by null models are actually related to competition between species and in which extent. For example, it is possible to test competition between leafhoppers by using some model species (selected from both polyphagous leafhoppers and most common plant species) and varying the species assemblages in terms of species identity and abundances. These experimental test are crucial in order to establish, e.g. if segregation is due to direct competition between species or to different environmental requirements.

Enhancement of biodiversity associated to vineyards

In Switzerland, ecological direct payments (subsidies) to promote a high level of biodiversity are only granted to vine-growers that satisfy a number of ecological requirements (Swiss Federal Ordinance on Direct Payments in Agriculture, OPD of 23 October 2013). Basically, a quality value for the vineyard is calculated by a monitoring scheme using a scored list of 59 non-productive plants belonging to the Red List or species of particular interest. However, it is widely accepted that the concept of biodiversity embraces two complementary components: taxonomic and functional diversity. Using a two-step multivariaate analyses approach, we identified indicator species that are significantly associated with high values of taxonomic and functional biodiversity. Out of 52 indicator species, 24 (46%) were exclusively selected by functional biodiversity indices whereas only 10 (19%) were associated with taxonomic indices. Eighteen (35% of the total) species were selected by both types of indices. To consider functional aspects of biodiversity in diversity-conservation strategies, we proposed a conceptual framework as a tool for the selection of suitable plant indicators. It is based on four criteria for the selection of indicator plant species: 1) Management intensity, 2) Components of biodiversity, 3) Vulnerability and threat of extinction, 4) Real and potential harm to biodiversity. Applying the framework to the vineyards of Southern Switzerland allowed to select a total of 118 species. These where associated with low management intensities, high biodiversity values, vulnerable species and species threat of extinction, and a high degree of harm to biodiversity. We consider, the list of selected indicator species for the vineyard agroecosystem in the Southern Swiss Alps as a basic tool for calculating the quality value to monitor the biodiversity in vineyard. Nevertheless, it would be desirable to apply the conceptual framework we proposed for the selection of indicators from taxa of different trophic levels and to integrate them in biomonitoring programs. We support a multi-taxa indicator approach with the aim to cover different ecological predictors (e.g. pollution, habitat types, climatic factors, soil and air properties) to which the taxon is particularly responsive.

Biodiversity conservation and phytosanitary measures

Positive impacts of biodiversity on cropland have been widely documented. In addition to providing the primary production upon which food chains are built, plants are key components for ecosystems functioning, e.g. by provision of cover, reproduction sites, structure within habitats, non-host foods such as pollen, nectar, alternative hosts and prey, and shelter (Marshall *et al.*, 2003). Arthropods contribute significantly to vital agro-ecological functions including biological pest control and as a food source for higher trophic levels (Losey and Vaughan, 2006). While agro-environmental measures are designed to protect all aspects of biodiversity, there are cases where some components of biodiversity associated to cropland can become an threat for productivity and stability of farming (e.g. pathogens, insect vectors, and weeds). In the last chapter, we present the case of vector-borne grapevine diseases (Flavescence dorée-FD and and Bois Noir-BN) and caused by phytoplasma, some of which are quarantine pathogens. The disease control strategies are mainly focused on prevention, and in case of FD-phytoplasma on mandatory application of insecticides, which are not always effective. Despite phytosanitary measures, phytoplasma diseases continue to spread in all countries, suggesting that the epidemiology of the diseases is still not completely understood and this chemical warfare is a failure. In this study, we detected the presence of two confirmed and three suspected Auchenorrhyncha vectors inhabiting the vineyards in southern Switzerland. About the known vector (*Scaphoideus titanus*) of quarantined phytoplasma, an high prevalence of infection in insect body was still observed locally despite mandatory control programs. We conclude that other leafhoppers could play a role in spreading the disease (e.g. *Orientus ishidae*). Prevalence of infection of *O. ishidae* was high, both at regional (27 %) and local scale (ranging from 14 to 100 %). Although the detection of phytoplasma in an insect body does not necessarily prove its vector status, this detailed screening allowed us to get a clearer vision on the spread of grapevine phytoplasmas in the vineyard agroecosystem. A regional scale approach is very important for disentangling the complex issue of grapevine phytoplasma diseases, to provide background information for both vectors and phytoplasmas, and to understand the possible spread of the disease. According to our findings, we support further investigations covering both basic knowledge on the vector status of suspected vectors and the implementation of sustainable management strategies towards a mutual interest to protect crop yield and ecosystem functioning.

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Scherr S.J. and McNeely J.A. 2008. Biodiversity conservation and agricultural sustainability: towards a new paradigm of 'ecoagriculture' landscapes. Phil. Trans. R. Soc. B, 363, 477–494.

Curriculum Vitae

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EDUCATION

PhD in Biology **26.04.2016**
University of Neuchâtel (CH) and WSL (CH); Advisor: Prof. Edward A.D. Mitchell, co-advisor: Dr. Marco Moretti.
Thesis: Biodiversity conservation and sustainable management in the vineyard agroecosystem: an integrated approach for different trophic levels

M.Sc. in Environmental Science (specialization in agro-forestry), *summa cum laude* **04.2003**
University of Pisa (IT); Advisor: Prof. Luciano Santini
Thesis: Observations on arthropods in two vineyards with different management inputs

RESEARCH AND PROFESSIONAL EXPERIENCE

Responsible Swiss Applicant **04.2014-Present**

SCOPES programme of the SNSF (01.04.2014 – 01.04.2017); http://p3.snf.ch/project-152414, European Eastern partner Serbian team headed by Dr. Milana Mitrović.

Project: Epidemiology and management strategy of Stolbur phytoplasma in agroecosystems.

- Coordinating the Swiss team
- Organised and carried out field sampling and laboratory trials
- Compiling results: 1 article, two conference paper
- Managed administrative issues

Doctoral Researcher **04.2012-04.2016**

University of Neuchâtel (CH) and WSL

Project granted by Federal Office for the Environment.

- Major Results: defined a three-step analysis to evaluate species coexistence in a two trophic-level system; defined a two-step multivariate analysis approach to sort indicator plant species for different aspects of biodiversity; conceived a conceptual framework to select reliable indicator species based on different biodiversity conservation criteria; detected two potential vectors of pathogens causing grapevine yellow diseases in Switzerland.
- Four first-author articles have been accepted and 1 article in submission.
- 7 additional scientific papers, 6 divulgative articles and 7 conference presentations.

Advanced Postgraduate Fellow **01.2014-12.2015**

Project granted by DECS, Department "Educazione Cultura e Sport" of Ticino Canton (CH)

Title: The leafhoppers of vineyards: pathogens, potential vectors and their parasitoids

- Characterization of Leafhopper and Planthopper community in vineyards South of Swiss Alps
- Defined potential leafhopper vectors of phytoplasmas causing grapevine diseases
- Characterization of fairyfly (Hymenoptera: Mymaridae) communities in vineyards
- Recorded new species of leafhoppers, plantoppers and fairyfly in Switzerland

Entomology Specialist **2012-2015**

Professional services (one month per year) founded by Agroscope Changins-Wädenswil (ACW) Center of Cadenazzo (CH), in the frame of two different internal project.

- Identification of Auchenorrhyncha specimens collected in vineyards and on woody plants, with particular attention to known and potential vectors of phytoplasma
- Identification of Auchenorrhyncha specimens collected on commercial fields of aromatic plants

Entomology Specialist **02.2014-05.2014**

Professional services founded by WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf (CH), in the frame of SNF project: Exclosure Experiment in the Swiss National Park, 2009-2014

- Identification of Auchenorrhyncha collected in alpine grasslands and measurement of morphological characters of specimens.
- Co-authorship in a ISI-paper

Entomology Specialist **2008-2012**

Professional services (one month per year) founded by Natural History Museum (MCSN), Lugano (CH)

- Identification of Auchenorrhyncha specimens collected in five different habitat: peat bogs, city gardens, Botanical Gardner, wetland areas, vineyard.
- Created a reference collection of the Hemiptera Fulgoromorpha and Cicadomorpha (Auchenorrhyncha) preserved in MCSN

Research Assistant **09.2010-08.2011**

WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Bellinzona (CH); Advisor: Dr. Marco Moretti

- Analysed biological data from a pilot survey on Biodiversity in vineyards in Tessin

Technician-scientific auxiliary **05.2010-03.2012**

Agroscope Changins-Wädenswil (ACW) Center of Cadenazzo (CH); Advisor: Dr. Mauro Jermini

Project funded by Commission for Technology and Innovation (CTI)
- Monitoring and experimental sampling of insect vectors in vineyard agroecosystem
- Tested enumerative and sequential sampling plans for *S. titanus* juvenils.

Postgraduate Fellow **09.2004-12.2007**
Agrobiology and Pedology Research Centre (IT); Advisor: Dr. Bruno Bagnoli

National Project founded by Council for Agricultural Research and Economics (IT)
- Studied the bio-ecology of know and potential vectors of phytoplasma
- Proving the vector capability of a potential vector to inolulate two phytoplasma strains in an artificial feeding medium
- Published one first-author article

Postgraduate Fellows **04.2003-09.2003**
04.2004-09.2004
University of Pisa (IT); Advisor: Prof. Andrea Lucchi

- Sampling and Rearing of juveniles forms and adults of Auchenorryncha
- Monitoring of insects (mainly Homoptera and Hymenoptera Parasitica) in natural and agricultural ecosystems of coastal Tuscany area

ACADEMIC AND RESEARCH TRAINING

- “Career Planning for Scientists”, two days, Fix the Leaky Pipeline Program **2015**
- “Theories and Methods in spatial community modelling”, four days, doctoral course **2015**
- “Successful fund acquisition for Researchers”, two days, WSL **2015**
- “Training in ADE4 in R, Module I: Basic Methods”, two days, doctoral course **2014**
- “An Introduction to R”, two days, doctoral course **2014**
- “R course in community assembly analyses”, three days, Tartu, Estonia **2013**
- "Systematic reviews and meta-analyses in ecology”, two days, doctoral course **2012**
- “23° Course of Statistic Methodology in basic and applied Biological Research”, five days, Massa Carrara, Italy **2007**

SKILLS AND TECHNIQUES

General skills in research project management and data analysis. Specific expertise and interests in:

Biological Skills
- broad knowledge of entomology
- specialist on identification of Auchenorrhyncha species
- insect rearing
- isolation of DNA

Computing Skills
- Microsoft Office Suite, Internet Explorer
- Graphical applications: Paint.net, photoshop
- ArcGis, QGis, Endnote

Others Skills

- Statistical software: R, Sigmaplot, STATISTICA, Canoco, Syntax
- Data and information collection
- Writing and presenting reports

Social Skills: I have increased my social skills by collaborating in many researches group from different Cantons and abroad too.

Organizational Skills: I have trained my organizational ability coordinating some personnel employed on Pilot and BioDiVine projects.

Technical skills and competences: I have over 9 years' experience working on sampling methodology of insects, so I consider my knowledge on this topic to be very good.

TEACHING EXPERIENCE

- Substitute teacher, High School of Gardener, Trevano, Switzerland, 2010
- Guest lecturer, Bayer CropScience, Acquaviva di Montepulciano (IT), 2005
- Seminar course to technician on "The Flavescence dorée and its vector *Scaphoideus titanus* in Central Italy"

SOCIETY MEMBERSHIP

- Association "Arbeitskreis Zikaden Mitteleuropas" (Study group leafhoppers and planthoppers of Central Europe)
- Society of Natural Science of Ticino, Switzerland
- Pronatura

COMMUNICATIONS

https://www.researchgate.net/profile/Valeria_Trivellone/publications

ISI-Papers

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5. **Trivellone V.,** Schönenberger N., Bellosi B., Jermini M., de Bello F., Mitchell E. and Moretti M. 2014. *Indicators for taxonomic and functional aspects of biodiversity in the vineyard agroecosystem of Southern Switzerland.* Biological Conservation. 170:103-109.
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29. Alma A., Lessio F., Picciau L., Tota F., Forte V., Borgo M., Bagnoli B., Pinzauti F., **Trivellone V.**, Rapisarda C., Cavalieri V., D'Urso V. 2008. *Rapporti tra cicaline, fitoplasmi e piante ospiti nell'agroecosistema vigneto.* Petria, 18 (2): 257-260.
30. Bagnoli B., Pinzauti F., **Trivellone V.** 2005. *Indagine preliminare sugli auchenorrinchi potenziali vettori di stolbur in un'area viticola del Lazio.* Petria, 15 (1/2): 55-58.
31. Alma A., Lessio F., Pavan F., Forte V., Angelini E., Borgo M., Bagnoli B., Pinzauti F., **Trivellone V.** 2005. *I giallumi della vite: un fattore limitante le produzioni vitivinicole. Rilevamento di auchenorrinchi vettori accertati e potenziali di fitoplasmi.* Petria, 15 (1/2): 151-153.

Conference Proceedings and Talks - (*oral presentation given by myself)

32. **Trivellone V.*,** Jermini M., Angelini E. 2015. *Occurrence of Leaf- and Planthoppers known and potential vectors of phytoplasmas in vineyards of Southern Switzerland*. IOBC-WPRS Conference of Working Group on "Integrated Protection and Production in Viticulture", Vienna, October 2015. Abstract Book and talk.
33. **Trivellone V.,** Jermini M., Posenato G., Mori N. 2015. *Influence of pruning wood management and suckering on Scaphoideus titanus Ball density in two distinct wine-growing area.* IOBC-WPRS Conference of Working Group on "Integrated Protection and Production in Viticulture", Vienna, October 2015. Abstract Book.
34. **Trivellone V**. *, Filippin L, Jermini M., Angelini E. 2015. *Molecular characterization of phytoplasma strains in leafhoppers inhabiting the vineyards agroecosystem in Southern Switzerland.* 3rd International Phytoplasmologist Working Group Meeting. Mauritius, January 2015. In: Phytophatogenic Mollicutes, 5(1): S45-S46. DOI: 10.5958/2249-4677.2015.00018.3 and talk.
35. Mitrovic M., **Trivellone V.** *, Jovic J., Cvrkovic T., Jakovljevic M., Kosovac A., Krstic O., Toševski I. 2015 *Potential Hemipteran vectors of "stolbur" phytoplasma in potato fields in Serbia*. 3rd International Phytoplasmologist Working Group Meeting. Mauritius, January 2015. In: Phytophatogenic Mollicutes 5(1):S49-50. DOI:10.5958/2249-4677.2015.00020.1 and talk.
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43. Prevostini M., Taddeo A., Balac K., **Trivellone V**., Rigamonti I., Baumgärtner J., Jermini M. 2011. *WAMS - an adaptive system for knowledge acquisition and decision support: the case of* Scaphoideus titanus. IOBC-WPRS Bulletin 85:
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45. **Trivellone V.**, Pinzauti F., Bagnoli B. 2006. Reptalus quinquecostatus *(Dufour) (Cixiidae): potenziale vettore di stolbur in un ambiente viticolo toscano.* XXI Congresso Nazionale Italiano di Entomologia, Campobasso, 11-16/06/2007, Proceedings, p. 174.

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47. Pezzatti B., Cara C., Milani L., Trivellone V., Müller F., Moretti M., Jermini M. 2013. *Factors affecting the parasitoid complex of* Phyllocnistis vitegenella *Clemens in vineyards of Southern Switzerland*. IOBC/WPRS Meeting of the Working Group "Integrated Protection and Production in Viticulture", Ascona (Switzerland), 13th - 17th October, 2013.
48. Jermini M., Gusberti M., **Trivellone** V., Wyss E. , Linder Ch. 2009. *Gebrauch biologischer Insektizide im Kampf gegen den* Scaphoideus titanus*, den Vektor von* Flavescence dorée. Pflanzengesundheit, Insektenregulierung, Poster: 314-317.

INVITED TALKS

- Giornata del viticoltore. Bellinzona, October 15th, 2015. Oral presentation: Trivellone V. *Aumentare la qualità ecologica nei vigneti: un approccio integrato*
- DEG-Seminar. WSL, Birmensdorf, November 26th, 2014. Oral presentation: Trivellone V. *Approaches to biodiversity conservation and sustainable management in the vineyard agroecosystem.*
- Giornata del viticoltore. Pregassona, December 12th, 2009. Oral presentation: Trivellone V. *Studio sulla Biodiversità dell'Artropodofauna in vigneti Ticinesi, con particolare riferimento alla comunità di Cicaline (Auchenorrinchi)*.
- Seminario tecnico organizzato da ARSIA Regione Toscana, ARPAT e Provincia di Massa Carrara su "Flavescenza dorata della vite in Toscana: situazione, controllo e assistenza tecnica". Massa, 24 Marzo 2005 (Bagnoli B., Pinzauti F., Trivellone V. Scaphoideus titanus: *riconoscimento, biologia e controllo*).

TECHNICAL REPORTS

- Trivellone V., Bellosi B., Moretti M. Criteri per la valutazione della qualità per la biodiversità dei vigneti a Sud delle Alpi della Svizzera. Report number: Sezione Agricoltura (RSA14068 del 24.11.2014) and Ufficio Natura e Paesaggio, Sezione dello sviluppo territoriale (Rif. 772-43/2014 del 23.04.2014). Affiliation: WSL- Swiss Federal Institute for Forest, Snow and Landscape Research
- Trivellone V., Pollini Paltrinieri L., Schönenberger N., Jermini M., Moretti M. Progetto BioDiVine – Biodiveristà, qualità biologica e conservazione delle specie nell'agroecosistema vigneti. Report number: 06.0127.PZ/L21 1-1 867. Affiliation: Consorzio BioDiVine.
- Trivellone V. Studio della biodiversità della Auchenorrincofauna in vigneti ticinesi. Affiliation: Agroscope Changins-Wädenswil.

REFERENCES

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