Assessing the Effects of Land-use Change on Plant Traits, Communities and Ecosystem Functioning in Grasslands: A Standardized Methodology and Lessons from an Application to 11 European Sites

ERIC GARNIER1,*, SANDRA LAVOREL2, PAULINE ANSQUER3, HELENA CASTRO4, PABLO CRUZ3, JIRI DOLEZAL5, OVE ERIKSSON6, CLAIRE FORTUNE1, HELENA FREITAS4, CARLY GOLODETS7, KARL GRIGULIS2, CLAIRE JOUANY3, ELENA KAZAKOUM, JAIME KIGEL7, MICHAEL KLEYER8, VEIKO LEHSTEN8, JAN LEPŠ3, TONIA MEIER8, ROBIN PAKEMAN9, MARIA PAPADIMITRIOU10, VASILIOS P. PAPANASTASIS10, HELEN QUESTED6, FABIEN QUÉTIER2, MATT ROBSON2, CATHERINE ROUMET1, GRACIELA RUSCH11, CHRISTINA SKARPE11, MARCELO STERNBERG12, JEAN-PIERRE THEAU3, AURELIE THÉBAULT2,†, DENIS VILE1,13,‡ and MARIA P. ZAROVALI10

1CNRS, Centre d’Ecologie Fonctionnelle et Evolutive (UMR 5175), 1919 route de Mende, 34293 Montpellier Cedex 5, France, 2Laboratoire d’Ecologie Alpine (CNRS UMR 5553) and Station Alpine Joseph Fourier (UMS-UJF-CNRS 2925), Université Joseph Fourier, BP 53, F-38042 Grenoble, Cedex 09, France, 3UMR 1248 ARCHE, Équipe ORPHEE, INRA, BP 52627, 31326 Castanet-Tolosan, France, 4Department of Botany, University of Coimbra, 3000 Coimbra, Portugal, 5University of South Bohemia, Faculty of Biological Sciences, Botany Department, Na Sadkach 7, Ceske Budejovice 37005, Czech Republic, 6Department of Botany, Stockholm University, Stockholm, 106 91, Sweden, 7Institute of Plant Sciences and Genetics in Agriculture, Faculty of Biological, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel, 8Landscape Ecology Group, University of Oldenburg, 26111 Oldenburg, Germany, 9The Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, UK, 10Laboratory of Rangeland Ecology, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece, 11Norwegian Institute for Nature Research, Tungasletta 2, 7485 Trondheim, Norway, 12Department of Plant Sciences, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel and 13Département de Biologie, Université de Sherbrooke, Sherbrooke (QC), Canada J1K2R1

Received: 29 May 2006 Returned for revision: 25 July 2006 Accepted: 24 August 2006 Published electronically: 3 November 2006

Background and Aims A standardized methodology to assess the impacts of land-use changes on vegetation and ecosystem functioning is presented. It assumes that species traits are central to these impacts, and is designed to be applicable in different historical, climatic contexts and local settings. Preliminary results are presented to show its applicability.

Methods Eleven sites, representative of various types of land-use changes occurring in marginal agro-ecosystems across Europe and Israel, were selected. Climatic data were obtained at the site level; soil data, disturbance and nutrition indices were described at the plot level within sites. Sixteen traits describing plant stature, leaf characteristics and reproductive phase were recorded on the most abundant species of each treatment. These data were combined with species abundance to calculate trait values weighted by the abundance of species in the communities. The ecosystem properties selected were components of above-ground net primary productivity and decomposition of litter.

Key Results The wide variety of land-use systems that characterize marginal landscapes across Europe was reflected by the different disturbance indices, and were also reflected in soil and/or nutrient availability gradients. The trait toolkit allowed us to describe adequately the functional response of vegetation to land-use changes, but we suggest that some traits (vegetative plant height, stem dry matter content) should be omitted in studies involving mainly herbaceous species. Using the example of the relationship between leaf dry matter content and above-ground dead material, we demonstrate how the data collected may be used to analyse direct effects of climate and land use on ecosystem properties vs. indirect effects via changes in plant traits.

Conclusions This work shows the applicability of a set of protocols that can be widely applied to assess the impacts of global change drivers on species, communities and ecosystems.

Key words: Climate gradient, disturbance, ecosystem properties, European marginal agriculture, land-use change, methods, nutrient limitation, plant community, plant functional traits, soil properties.

INTRODUCTION

A conceptual framework to understand the links between species and ecosystem functioning using plant traits was recently proposed by Chapin et al. (2000) and further refined (Díaz and Cabido, 2001; Lavorel and Garnier, 2003). This framework suggests that species traits, such as growth rate, competitive ability, and resource use, reflect species adaptations to environmental conditions. Recent research has focused on understanding the functional organization of plant communities and the responses of ecosystems to global change drivers. One key aspect of this research is the development of standardized approaches to assess the impacts of land-use change on plant traits, communities, and ecosystem functioning.

In this study, we present a standardized methodology to assess the effects of land-use change on plant traits, communities, and ecosystem functioning in grasslands. The methodology assumes that species traits are central to these impacts and is designed to be applicable in different historical, climatic contexts, and local settings. It is based on the use of standardized protocols to measure plant traits and ecosystem properties, and the analysis of these data to identify patterns of change associated with land-use modifications.

The methodology was applied to 11 European sites, representing various types of land-use changes occurring in marginal agro-ecosystems across Europe and Israel. The sites were selected based on their representativeness of different land-use systems, and they were characterized by a wide range of climatic, environmental, and soil conditions. The sites were visited to collect data on climatic, soil, and disturbance factors, as well as on plant traits and ecosystem properties.

The plant traits measured included vegetative plant height, leaf characteristics, and reproductive phase. These traits were recorded for the most abundant species of each treatment, and their values were weighted by the abundance of species in the communities. The ecosystem properties selected were components of above-ground net primary productivity and decomposition of litter. The data were analyzed to identify patterns of change associated with land-use modifications.

The results showed that the wide variety of land-use systems characterized marginal landscapes across Europe was reflected by the different disturbance indices and soil and/or nutrient availability gradients. The trait toolkit allowed us to describe adequately the functional response of vegetation to land-use changes, but some traits, such as vegetative plant height and stem dry matter content, should be omitted in studies involving mainly herbaceous species. The study also demonstrated how the data collected can be used to analyze direct effects of climate and land use on ecosystem properties versus indirect effects via changes in plant traits.

The methodology presented in this study can be widely applied to assess the impacts of global change drivers on species, communities, and ecosystems. It is based on a standardized approach to measure plant traits and ecosystem properties, and the analysis of these data to identify patterns of change associated with land-use modifications. The methodology is designed to be applicable in different historical, climatic contexts, and local settings, and it has the potential to be refined and adapted to specific research questions.
It distinguishes ‘functional response traits’, which are species traits that vary consistently in response to changes in environmental factors, and ‘functional effect traits’, which are species traits that feed back to ecosystem functioning. The main hypothesis put forward by Lavorel and Garnier (2002) was that traits involved in resource acquisition and use at the species level would scale-up to ecosystem functioning, provided that traits are weighed by the species’ contribution to the community. This is known as the ‘biomass ratio hypothesis’ (Grime, 1998). Based on a list of plant traits derived from Weiher et al. (1999), we designed a multi-site test of this virtually untested hypothesis (but see Garnier et al., 2004; Qué-tier et al., 2006), in the context of changes in land use occurring and projected in marginal agro-ecosystems within Europe (Klein Goldewijk, 2001; Rounsevell et al., 2006). This was conducted as part of the project ‘Vulnerability of Ecosystem Services to Land Use Change in Traditional Agricultural Landscapes’ (VISTA), funded by the European Union over the period 2003–2005.

The objectives were to: (1) identify changes in species traits in response to modifications of land use, and (2) use plant traits to scale-up from the functioning of species to that of populations and ecosystems, using easily measurable functional traits, in a large range of situations in terms of both climate (dry, wet, warm, cold) and types of land-use changes (abandonment, reduction of grazing pressure or fertilization, etc.). It was therefore a prerequisite to standardize as far as possible the design and data collection across the different sites. McIntyre et al. (1999) persuasively argued that valuable inter-site and inter-study comparisons on trait response require standardization of trait and disturbance measurement, but here, the questions addressed required standardization on an even wider array of variables. The main aim of this paper is to describe the standardized methods developed in the framework of this multi-site study designed to assess the impacts of land-use change on vegetation. It covers aspects pertaining to (1) the measurement of environmental variables (soil and climate), plant traits at the species and whole community levels and ecosystem properties; (2) data organisation; and (3) data analyses. Some preliminary results obtained across sites are presented to demonstrate the applicability of this methodology. We then discuss methodological opportunities and constraints, as well as the scope of this methodology for worldwide analyses of plant functional responses and ecosystem effects of land-use change.

MATERIAL AND METHODS

Overview of the experimental design

The study was conducted at ten sites across Europe and one site in Israel (Table 1). Within each site, a number of land-use regimes were identified (Table 1), considered as ‘treatments’ hereafter. These treatments can be classified into three main categories: extensification due to a reduction of grazing pressure, a decrease in fertilizer input or complete land abandonment. For each land-use regime, a number of (in most cases) non-contiguous replicate plots were selected in the landscape. The exact design was dependent on the spatial arrangement of the land in each site. Within each plot, sampling areas (‘subplots’) were selected to collect community composition data, species traits and ecosystem properties (see below). On average, there were 4.5 treatments per site (range: 3–8), with four replicate plots per treatment (range: 2–7), giving a total of 48 treatments and 194 plots across the 11 sites (Table 1).

Environmental variables at site level

Climate. Monthly data of temperature and rainfall were obtained from the meteorological stations closest to the sites, and solar radiation data were obtained from satellite (http://www.satellight.com/). In order to relate the response of traits/ecosystem functions across the 11 sites to variations in climate, a series of synthetic climate indices have been developed, describing site climates as a linear function of combined climate parameters. Calculated indices include potential evapotranspiration (PET, calculated after Hargreaves and Samani, 1985), growing degree-days (GDD: e.g. Wang, 1960), simple indexes of rainfall effectiveness (rainfall compared with PET) and the aridity index of Thornwaite (1948), which reflects monthly rainfall deficits (PET greater than rainfall) as a proportion of annual PET.

Environmental variables at plot level

Soil data. Soil samples (0–5 cm horizon) were collected at the end of the winter period with a hand-auger, after the upper root mat was discarded if present. In each plot 10–20 cores, evenly distributed on the whole plot area, were collected and bulked to make a composite sample representative of the plot. Soils were crumbled by hand and dried for 1 week at room temperature, then sieved at 2 mm before analysis. All soils samples were analysed by the ‘Laboratoire d’Analyses des Sol’ of the National Institute for Agronomic Research (INRA, Arras, France) using standard procedures (Afnor, 1994). The following variables were measured (Table 2): pHwater, texture (sand, loam clay), CaCO3, total C and N concentrations; the plant available P fraction was determined by the Olsen procedure (Olsen et al., 1954). Water-holding capacity was derived from these raw data, using a modified version of the equation provided by Saxton et al. (1986; see http://www.bsyse.wsu.edu/saxton/).

Disturbance regimes. The parameterization of disturbance regimes across the VISTA sites and land-use types builds on disturbance descriptors developed by White and Pickett (1985), Kleyer (1999), and White and Jentsch (2001). Five parameters were used to describe the disturbance regime: (1) pre-treatment regime (land-use category), (2) type of disturbance (land-use category), (3) return interval of disturbance (year), (4) onset of disturbance (Julian day in the year) and (5) intensity of disturbance (percentage biomass removed). In case of rotational disturbances, each disturbance was parameterized separately and the rotation was characterized by aggregating the single values.
<table>
<thead>
<tr>
<th>Country</th>
<th>Name (abbreviation)</th>
<th>Coordinates</th>
<th>Altitude range (m)</th>
<th>Type of climate</th>
<th>Temperature (°C)</th>
<th>Rainfall (mm)</th>
<th>Land-use change</th>
<th>Primary disturbance</th>
<th>No. of treats/plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Israel</td>
<td>Karei Deshe (IS-KDE)</td>
<td>32°35'N, 32°35'E</td>
<td>150</td>
<td>Mediterranean semi-arid</td>
<td>19.6</td>
<td>572</td>
<td>Abandonment/extensification</td>
<td>Grazing</td>
<td>4/8</td>
</tr>
<tr>
<td>Portugal</td>
<td>Mértola/Castro Verde (PT-MER)</td>
<td>37°40'N, 8°00'E</td>
<td>100–150</td>
<td>Mediterranean</td>
<td>16.6</td>
<td>538</td>
<td>Extensification/abandonment</td>
<td>Grazing/mowing</td>
<td>4/12</td>
</tr>
<tr>
<td>Greece</td>
<td>Lagadas (GR-LAG)</td>
<td>40°47'N, 23°12'E</td>
<td>450–550</td>
<td>Mediterranean semi-arid</td>
<td>12.1</td>
<td>586</td>
<td>Extensification/abandonment</td>
<td>Grazing/ploughing/cutting</td>
<td>4/16</td>
</tr>
<tr>
<td>France</td>
<td>Hautes Garrigues du Montpellierais (FR-HGM)</td>
<td>43°51'N, 3°56'E</td>
<td>100–160</td>
<td>Mediterranean sub-humid</td>
<td>13.2</td>
<td>994</td>
<td>Abandonment</td>
<td>Perennial crops</td>
<td>3/12</td>
</tr>
<tr>
<td>Germany</td>
<td>Müritz National Park (GE-MNP)</td>
<td>53°27'N, 12°44'E</td>
<td>65</td>
<td>Temperate</td>
<td>8.7</td>
<td>639</td>
<td>Extensification/abandonment</td>
<td>Grazing</td>
<td>8/56</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>Ohrzeni (CZ-OHR)</td>
<td>48°57'N, 14°36'E</td>
<td>510</td>
<td>Central-European temperate</td>
<td>8.2</td>
<td>583</td>
<td>Experimental regimes</td>
<td>Mowing</td>
<td>4/12</td>
</tr>
<tr>
<td>Sweden</td>
<td>South-east Baltic Sea (SE-BAL)</td>
<td>58°50'N, 17°24'E</td>
<td>0–50</td>
<td>Cold temperate</td>
<td>6.0</td>
<td>551</td>
<td>Abandonment/extensification</td>
<td>Crops/mowing/mowing</td>
<td>4/20</td>
</tr>
<tr>
<td>UK (Scotland)</td>
<td>South Uist (Stauningbrig) (SC-SUT)</td>
<td>57°16'N, 7°24'E</td>
<td>0–15</td>
<td>Oceanic</td>
<td>8.4</td>
<td>1275</td>
<td>Abandonment</td>
<td>Rotational agriculture</td>
<td>3/9</td>
</tr>
<tr>
<td>France</td>
<td>Ercé (FR-ERC)</td>
<td>42°50'N, 1°17'E</td>
<td>600–1000</td>
<td>Mountain humid</td>
<td>10.0</td>
<td>1079</td>
<td>Extensification</td>
<td>Grazing/mowing</td>
<td>6/18</td>
</tr>
<tr>
<td>France</td>
<td>Col du Lautaret (FR-LAU)</td>
<td>45°02'N, 6°21'E</td>
<td>1900–2100</td>
<td>Sub-alpine</td>
<td>3.0</td>
<td>902</td>
<td>Extensification</td>
<td>Mowing/grazing/ploughing</td>
<td>5/15</td>
</tr>
<tr>
<td>Norway</td>
<td>Bøttsjøndalen/ Berghøgda (NO-BER)</td>
<td>62°42'N, 11°05'E</td>
<td>800–900</td>
<td>Alpine</td>
<td>0.6</td>
<td>750</td>
<td>Grazing intensity/Abandonment</td>
<td>Grazing</td>
<td>4/16</td>
</tr>
</tbody>
</table>

Temperature and rainfall are mean annual values, usually taken over 30-year periods. The numbers of treatments (treats) and plots in each site are given in the last column.
Nutrition indices. Nitrogen (NNI) and phosphorous (PNI) indices were used to determine nutrient limitation for plant growth. NNI was calculated as the ratio between the actual nitrogen concentration of above-ground biomass and the critical nitrogen concentration (i.e. concentration allowing potential growth), as proposed by Lemaire, (1997). PNI, which depends on nitrogen concentration of above-ground biomass, was calculated as proposed by Duru and Ducrocq (1997) and Jouany et al. (2004). Formulas and details for both indices are given in Appendix 1.

Species and traits

Species selected for trait measurements were those most abundant species that collectively made up at least 80% of the maximum standing live biomass of the community. This threshold has been suggested to ensure a satisfactory description of community properties in relation to biogeochemical cycles in ecosystems (cf. Garnier et al., 2004; Pakeman and Quested, 2006). Two types of data were collected on these species. (1) The first were characteristics of the taxa that were considered as invariant across sites. These are: species names, botanical family, type of reproduction, life form, photosynthetic pathway, nutrient uptake strategy, mycorrhizal type and Ellenberg figures for tolerance to different ecological factors. These were collected from local floras and reference books, while species name followed the taxonomic nomenclature given by the Euro+Med PlantBase (http://www.euromed.org.uk/). (2) The second type were 16 traits – 11 quantitative, five categorical – which were measured in each of the 11 sites (Table 3). These traits were selected for their known or assumed responses to the factors studied, in particular disturbance regime and/or level and nutrient availability (see numerous examples and references in, for example, Chapin et al., 1993; Lavorel and Cramer, 1999; Grime, 2001; Lavorel and Garnier, 2002; Pausas et al., 2003). For most of them, the chosen protocols and the number of replicates measured followed Cornelissen et al. (2003). Protocols had to be refined for three traits: clonality, pollination mode and grazing defence (categories available upon request).

Based on previous studies comparing intra- vs. interspecific variability of these traits (e.g. Garnier et al., 2001; Roche et al., 2004; Fenner and Thompson, 2005, and references therein), these were grouped into either ‘variable’ or ‘stable’ traits among treatments (Table 3). For variable traits, replicates were taken within the different subplots of a particular treatment; the trait value for this treatment was then the average over the samples taken from all the different subplots. For stable traits, replicates were taken in a sample of the different subplots across the different treatments; the trait value for all treatments was then the average over the samples taken from the different subplots across all treatments. Therefore, for stable traits, an average value per species was assigned for a site, while for variable traits, an average value per species per treatment was assigned.
Table 3. List of traits retained, abbreviations and units, if not categorical: six are known to be variable within species according to abiotic and/or biotic conditions, while the remaining ten are considered stable within species across treatments. If a particular species is found in more than one treatment at a given site, variable traits are therefore measured in each treatment for this species, while stable traits are measured only once at a given site.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Abbreviation</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable traits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonality</td>
<td>–</td>
<td>Categorical</td>
</tr>
<tr>
<td>Vegetative plant height</td>
<td>VPH</td>
<td>cm</td>
</tr>
<tr>
<td>Reproductive plant height</td>
<td>RPH</td>
<td>cm</td>
</tr>
<tr>
<td>Leaf nitrogen concentration</td>
<td>LNC</td>
<td>mg g⁻¹</td>
</tr>
<tr>
<td>Leaf phosphorous concentration</td>
<td>LPC</td>
<td>mg g⁻¹</td>
</tr>
<tr>
<td>Onset of flowering</td>
<td>OFL</td>
<td>day of year</td>
</tr>
<tr>
<td>Stable traits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life history</td>
<td>–</td>
<td>Categorical</td>
</tr>
<tr>
<td>Plant height from florals</td>
<td>FPH</td>
<td>cm</td>
</tr>
<tr>
<td>Specific leaf area</td>
<td>SLA</td>
<td>m² kg⁻¹</td>
</tr>
<tr>
<td>Leaf dry matter content</td>
<td>LDMC</td>
<td>mg g⁻¹</td>
</tr>
<tr>
<td>Stem dry matter content</td>
<td>SDMC</td>
<td>mg g⁻¹</td>
</tr>
<tr>
<td>Leaf carbon concentration</td>
<td>LCC</td>
<td>mg g⁻¹</td>
</tr>
<tr>
<td>Seed mass</td>
<td>SM</td>
<td>mg</td>
</tr>
<tr>
<td>Dispersal mode</td>
<td>–</td>
<td>Categorical</td>
</tr>
<tr>
<td>Pollination mode</td>
<td>–</td>
<td>Categorical</td>
</tr>
<tr>
<td>Grazing defences</td>
<td>–</td>
<td>Categorical</td>
</tr>
</tbody>
</table>

*At some sites, all traits were considered as ‘variable’, and were therefore measured in all treatments (see text for details).

Species abundance and aggregated plant traits

Species richness and abundance were determined from live biomass contribution, cover, frequency or point quadrats on transects, depending on the site and the vegetation present. From these, community-aggregated plant traits at peak standing biomass (i.e. at the time when the maximum live biomass of a given community was reached) were calculated. For continuous traits, this was done as:

\[ \text{trait}_{\text{agg}} = \sum_{i=1}^{n} p_i \times \text{trait}_i \]

where \( p_i \) is the relative contribution of species \( i \) to the community, and \( \text{trait}_i \) is the trait value of species \( i \). For categorical traits, the relative contribution of each particular attribute was calculated as the sum of relative abundances of species within that attribute. In both cases, this was calculated with species that collectively make up at least 80% of the maximum standing biomass of the community.

Ecosystem properties

The ecosystem properties selected for study (Table 2) are key components of the carbon and nitrogen cycles (Chapin et al., 2002). These are: minimum and maximum live standing and dead biomass (terminology after Scurlock et al., 2002), above-ground net primary productivity and specific above-ground net primary productivity (SANPP), rate of litter decomposition, measured both in the field and in microcosms under controlled conditions in the laboratory, or assessed by near infra-red spectrometry.

Net primary productivity (NPP) represents the major input of carbon and energy into ecosystems. It can be considered as an integrative variable of the functioning of the whole ecosystem, owing to its relationships with animal biomass, secondary productivity and nutrient cycling (McNaughton et al., 1989). Although unbiased estimates of NPP are extremely difficult to obtain (Roberts et al., 1993; Scurlock et al., 2002), simple methods may provide fairly good indicators of the general ranking of study sites by productivity (Scurlock et al., 2002). Among those, we chose that based on the difference between maximum and minimum live above-ground biomass (ANPP) for two reasons: (1) it is by far the most frequently reported productivity data in the literature (cf. Esser et al., 2000), allowing us to compare our own data on a very broad scale, and (2) the determination of biomass at two different times allows the computation of SANPP, which expresses productivity on a community biomass basis instead of a ground area basis (see Appendix 2). It was also important to separate live and dead biomass, so as to take into account the specific effects of dead material, as reported in the results section.

In order to separate litter quality effects from impacts on the decomposition environment and decomposer organisms, and hence to refine our understanding of trait–decomposition links, we adopted a three-stage approach (Appendix 2). First, whole community, above-ground vascular plant litter from selected plots at all sites was decomposed in microcosms under standard conditions. Second, a standard litter was decomposed in each plot. Finally, community litter was decomposed in situ, to integrate the combined role of the environment, soil organisms and litter quality.

Standardized protocols for these different measurements were developed. The methods used represent a trade-off between feasibility and biological meaning. Summaries and relevant abbreviations used in the following text are given in Appendix 2, and full documents are available upon request.

Database

A database was designed to standardize the information described above: climate, site characteristics (soil properties, location, altitude, etc.), botanical and ecological information on species, traits, community composition and ecosystem properties. In addition to the general characteristics for the 11 sites, the database currently contains botanical and ecological data for more than 900 taxa, amounting to more than 1000 values for each trait.

Data analyses

Climate. In order to characterize better the climate of the sites a principal components analysis (PCA) was used to investigate the relationships between the sites in terms of mean annual rainfall, mean annual temperature, mean annual PET, annual growing degree-days, mean annual
solar radiation and Thornwaite’s aridity index. Annual PET and solar radiation were removed after a covariance analysis indicated that they were highly correlated with, in particular, mean annual temperature. Linear projections of the PCA axes were also used as a synthetic climate variable.

**Botanical composition.** It was not possible to compare the botanical composition of the plots at the species level owing to insufficient overlap in species composition between sites. Instead it was compared at the family level, representing gross scale variation in biogeographical and environmental differences between sites and plots. The comparison was carried out using detrended correspondence analysis (DCA; Hill and Gauch, 1980) using CANOCO v.4 (ter Braak and Šmilauer, 1998) on the percentage contribution of each family to the total cover of higher plants.

**Aggregated traits and ecosystem properties.** The overall effects of land-use regime within sites on the ten continuous measured traits will be presented here (Table 4). One-way ANOVAs were used for tests in aggregated trait values and ecosystem properties across land-use treatments within sites.

Inter-site analyses of variation of traits (here LDMC treated as an example) and ecosystem properties (here AGTotDead treated as an example), as well as their patterns of co-variation were tested using restricted maximum likelihood methods (REML) with the statistical package GenStat® 8.1 (GenStat, 2005). In these analyses, site was used as a random term, and we tested effects of land use, climate (represented by simple variables or the first axis of the PCA on climatic variables) and their interactions, taken as fixed additive terms. In order to test for the effects of community-level traits (here LDMC) on ecosystem properties (here AGTotDead) the same approach was applied, using traits and their interactions with land use as fixed terms in the model.

In the results presented here we compare only the least and most intensively used treatments at each site, while keeping levels as comparable as possible across sites. ‘Most used’ was taken to correspond to ‘traditional use’, usually associated with medium fertility and high biomass removal. ‘Least used’ corresponded to abandonment of these practices. For sites where abandonment was complete, we chose to focus on comparable lengths of succession, using plots with intermediate duration of abandonment (>10 years) vs. recently abandoned sites. At all sites these plots still had a strong herbaceous component.

**SELECTED RESULTS**

**Climatic variables**

The 11 sites encompass a large amount of climatic variation. Not only do they differ in terms of total rainfall, temperature (both given in Table 1), solar radiation, etc., but they also vary considerably in the seasonality and periodicity of these climatic parameters. The first axis of the PCA on site climatic parameters (75.7% of the variation) was largely associated with a gradient of aridity coupled with site temperature and growing degree-days (Fig. 1). PCA axis 2 (22.1% of the variation) was overwhelmingly linked to annual rainfall, separating two patterns underlying low aridity: high rainfall and moderate temperatures year-round, as opposed to true mountain sites with modest rainfall but low evaporation due to low temperatures during rainy periods. The projections of the sites on the two axes make it possible to separate the following groups of sites: (1) a group of Mediterranean sites with high temperatures and aridity (GR-LAG, PT-MER and IS-KDE; site abbreviations given in Table 1) – FR-HGM is linked with this group by high mean annual temperature but is distinguished by higher rainfall and lower aridity; (2) a group of colder sites but still with relatively low rainfall and medium values of aridity index (GE-MNP, CZ-OHR, SE-BAL); (3) a group of sites with similar temperatures to the above, but characterized by high rainfall and thus low aridity (SC-SUT, FR-ERC); and (4) a group of true mountain sites (FR-LAU, NO-BER) characterized by low temperatures, a short growing season, low aridity and medium rainfall.

Based on these results we chose to use synthetic climate indices such as the aridity index and projections of sites on PCA axis 1 (Fig. 1) as covariates for analyses of response of traits and ecosystem properties to land use.

**Disturbance indices**

Disturbance indices of VISTA treatments reflect the wide variety of land-use systems that characterize marginal landscapes across Europe, as shown by the return interval and intensity of disturbances for the least and most used treatments in each site (Fig. 2). The site with the highest disturbance was a short-rotational crop/fallow field in Scotland (SC-SUT: short return interval with the highest intensity), and the lowest was a pasture in Sweden (SE-BAL: longest return interval without disturbance) abandoned for more than 60 years. Aggregated return time of disturbances (Fig. 2A) and biomass removal by disturbance (Fig. 2B) show that the GE-MNP, IS-KDE, PT-MER and SE-BAL sites all span a considerably large range on the disturbance gradient, because they cover situations from continuing, moderately intense management to advanced secondary succession. The magnitude in disturbance regimes is more restricted at the NO-BER, FR-ERC, FR-LAU and FR-HGM sites. Among these, FR-ERC treatments are highly disturbed, whereas the abandonment sequence at FR-HGM belongs to the lower end of the total VISTA disturbance gradient. All other sites are intermediate with respect to variation in either disturbance frequency or biomass loss.

**Vegetation**

The DCA (Fig. 3) revealed significant turnover at the family level across all the sites and plots for the first two axes of the ordination (axes lengths of 6.58, 5.88, 2.82 and 2.55, respectively). These first two axes also explained a substantial part of the variation (percentage of total inertia explained of 10.9 and 8.1 compared with 3.5 and
### Table 4. Summary of significant differences and direction of response in aggregated means of the ten measured continuous plant traits among treatments (summarized in column 2) in the 11 sites of the VISTA project (results of within-sites one-way ANOVAs)

<table>
<thead>
<tr>
<th>Site</th>
<th>Disturbance/resource treatments</th>
<th>VPH</th>
<th>RPH</th>
<th>Flower</th>
<th>Seed mass</th>
<th>SLA</th>
<th>LDMC</th>
<th>LNC</th>
<th>LCC</th>
<th>LPC</th>
<th>StDMC</th>
<th>% Traits significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS-KDE</td>
<td>Grazing/abandonment</td>
<td>nd</td>
<td>+</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>nd</td>
<td>*</td>
<td>38 (8)</td>
</tr>
<tr>
<td>PT-MER</td>
<td>Agriculture/grazing/abandonment</td>
<td>***</td>
<td>nd</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>88 (8)</td>
</tr>
<tr>
<td>GR-LAG</td>
<td>Grazing/abandonment</td>
<td>ns</td>
<td>**</td>
<td>***</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>***</td>
<td>*</td>
<td>60 (10)</td>
</tr>
<tr>
<td>FR-HGM</td>
<td>Abandonment</td>
<td>ns</td>
<td>***</td>
<td>**</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>70 (10)</td>
</tr>
<tr>
<td>GE-MNP</td>
<td>Grazing/abandonment/water</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>100 (9)</td>
</tr>
<tr>
<td>CZ-OHR</td>
<td>Mowing/fertilization</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>*</td>
<td>ns</td>
<td>***</td>
<td>*</td>
<td>80 (10)</td>
</tr>
<tr>
<td>SE-BAL</td>
<td>Grazing/abandonment</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>67 (9)</td>
</tr>
<tr>
<td>SC-SUT</td>
<td>Agriculture/abandonment</td>
<td>ns</td>
<td>nd</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>44 (9)</td>
</tr>
<tr>
<td>FR-ERC</td>
<td>Mowing/grazing/fertilization</td>
<td>**</td>
<td>nd</td>
<td>**</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>+</td>
<td>+</td>
<td>66 (9)</td>
</tr>
<tr>
<td>FR-LAU</td>
<td>Mowing/grazing/fertilization</td>
<td>***</td>
<td>**</td>
<td>a</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>*</td>
<td>80 (10)</td>
</tr>
<tr>
<td>NO-BER</td>
<td>Grazing</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>nd</td>
<td>0</td>
<td>0 (9)</td>
</tr>
<tr>
<td>% Sites significant</td>
<td></td>
<td>60 (10)</td>
<td>88 (8)</td>
<td>82 (11)</td>
<td>37 (11)</td>
<td>64 (11)</td>
<td>55 (11)</td>
<td>64 (11)</td>
<td>36 (11)</td>
<td>78 (9)</td>
<td>88 (8)</td>
<td>0 (9)</td>
</tr>
<tr>
<td>Across-sites</td>
<td>Most used/least used</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>90 (10)</td>
</tr>
</tbody>
</table>

The last column gives the percentage of traits showing significant differences among treatments within a site (the number in parentheses is the number of traits available at that site), and the penultimate row gives the percentage of sites in which a particular trait varies significantly among treatments (the number in parentheses is the number of sites for which the trait is available). The final row gives the results of a REML analysis contrasting the most and least used treatments across the 11 sites.

Abbreviations: VPH, vegetative plant height; RPH, reproductive plant height; Flower, time of flowering; SLA, specific leaf area; LDMC, leaf dry matter content; LNC, leaf nitrogen concentration; LCC, leaf carbon concentration; LPC, leaf phosphorus concentration; StDMC, stem dry matter content. ns, not significant; a, P < 0.10; *, P < 0.05; **, P < 0.01; ***, P < 0.001. nd, no data. +, increase; –, decrease in response to decreasing land use. Hump-shaped responses are indicated by +/− or −/+. 

Garnier et al. — Methodology to Assess Effects of Land-use Change

973
The ordination clearly showed that many of the sites were relatively similar in cover at the family level with a dominance of Poaceae, as well as Apiaceae, Asteraceae, Rosaceae, Caryophyllaceae and Plantaginaceae. At high values on Axis 1 were the Norwegian sites (NO-BER) characterized by substantial cover of Ericaceae and Salicaeae, as well as the late successional Swedish sites with high values for the Ericaceae. Low values on Axis 1 were associated with Mediterranean woodland and scrub-dominated plots, including the GR-LAG and PT-MER sites dominated by Cistaceae and Fagaceae (Axis 1 scores of approx. 3).

Responses of plant traits to land-use change: an overview

Continuous traits (aggregated values) can be grouped into three categories, according to their sensitivity to treatments across sites (Table 4): (1) traits that respond to treatments in most sites — reproductive plant height, stem dry matter content, leaf phosphorus concentration and flowering time; (2) traits that respond in half to two-thirds of the sites — specific leaf area, vegetative plant height, leaf nitrogen concentration and leaf dry matter content; and (3) traits that respond in approx. one-third of the sites — seed mass and leaf carbon concentration. The REML analysis across the 11 sites highlighted strong and consistent responses across sites for all traits except seed mass. Direction of change was overall consistent for all traits with a significant response. Decreasing land use resulted at the community level in dominance by taller plants (increased vegetative and reproductive height), plants with more conservative leaf syndromes (decreased SLA, LNC and LPC, increased LDMC associated with increased SiDMC), and delayed flowering phenology. These responses were overall strongly consistent across sites, and the few exceptions resulted from either canopy closure after woody colonization (e.g. Sweden, Greece) or specific soil conditions (water-logging in Germany).

Multivariate analyses conducted at four individual sites identified consistent patterns in variation in plant strategies. At the SC-SUT site, Pakeman and Small (2004) showed that land use was a significant predictor of plant traits as abandonment of rotational cultivation led to an increase in species with more conservative nutrient use, had later flowering and larger seed sizes. However, species composition did not recover in the direction expected from analysis of unploughed areas. Overall, these results were repeated at the French alpine site (FR-LAU), where cessation of fertilization or of mowing resulted in the dominance by taller plants with more conservative nutrient use and later flowering, although seed mass did not vary across land-use treatments (Quétier et al., 2006). A path analysis conducted on the data from the French Mediterranean site (FR-HGM) demonstrated that abandonment of vine cultivation led to the progressive replacement...
of species with small stature, high rates of resource capture and early flowering by species with the opposite characteristics, while seed mass did not show any significant trend with time after abandonment (Vile et al., 2006).

Finally, Lepsˇ (1999) demonstrated significant changes in species composition as a response both to fertilization and to cessation of mowing. Plant height was a very good predictor of species success under fertilization, as only tall plants were able to survive in competition.

Responses of ecosystem and soil properties to land-use change: an overview (Table 5)

Maximum above-ground live (AGBmass) and dead biomass (AGTotDead) were significantly different among treatments in ten out of the 11 sites, but the effects of treatments were not necessarily comparable for the two variables (Table 5). By contrast, owing to the very tight correlation between maximum AGBmass and ANPP within (data not shown) and across sites ($r = 0.89, P < 0.001, n = 170$), the effects of treatments on these two variables were identical. Treatments had significant effects on average community decomposition rate measured under standard conditions, when there was no interaction with environmental conditions, in six out of the 11 sites. This reflects a decline in litter quality (C. Fortunel et al., unpubl. res.), which is a major determinant of in situ decomposition rates (Swift et al., 1979; Lavelle, 1993). The results of the in situ experiment are more complex (data not shown), however, as changes in the decomposition environment within sites also play a major role.

There was also a very close relationship between soil C and N both within (not shown) and across ($r = 0.87, P < 0.001, n = 194$) sites, with the consequence that treatment effects were almost identical on the two variables (the SC-SUT site being the only exception). Interestingly, nitrogen limitation as detected by the NNI had little concordance with information given by soil N. Phosphorus availability to plants as detected by soil P-Olsen was significantly different among treatments in nine of the sites, and except for two sites (IS-KDE and FR-LAU), these differences were consistent with those found using the PNI.

A methodology to link changes in disturbance regime, plant traits and ecosystem properties

One of the methodologies that can be used to assess the effects of land-use change on the functional properties of the vegetation through plant traits and environmental variables consists in successive sets of general modelling analyses. We present here the case of leaf dry matter content and above-ground total dead biomass as an illustration. The approach consisted of first identifying the effects of climate and land-use factors on LDMC, and then testing
Table 5. Summary of significant differences and direction of response to decreasing land use in selected ecosystem, community and soil properties among treatments (summarized in column 2) in the 11 sites of the VISTA project (results of within-sites one-way ANOVAs)

<table>
<thead>
<tr>
<th>Site</th>
<th>Disturbance/resource treatments</th>
<th>AGBmass</th>
<th>AGTotdead</th>
<th>ANPP</th>
<th>K decay-lab (NIRS)</th>
<th>Soil C</th>
<th>Soil N</th>
<th>Soil P-Olsen</th>
<th>NNI</th>
<th>PNI</th>
<th>Species richness</th>
<th>% Properties significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS-KDE</td>
<td>Grazing/abandonment</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>80 (10)</td>
</tr>
<tr>
<td>PT-MER</td>
<td>Agriculture/grazing/abandonment</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>+</td>
<td>+</td>
<td>90 (10)</td>
</tr>
<tr>
<td>GR-LAG</td>
<td>Grazing/abandonment</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>***</td>
<td>90 (10)</td>
</tr>
<tr>
<td>FR-HGM</td>
<td>Abandonment</td>
<td>–</td>
<td>/</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>/</td>
<td>/</td>
<td>70 (10)</td>
</tr>
<tr>
<td>GE-MNP</td>
<td>Grazing/abandonment/water</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>nd</td>
<td>nd</td>
<td>***</td>
<td>***</td>
<td>100 (8)</td>
</tr>
<tr>
<td>CZ-OHR</td>
<td>Mowing/fertilization</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td>a</td>
<td>***</td>
<td>***</td>
<td>–</td>
<td>60 (10)</td>
</tr>
<tr>
<td>SE-BAL</td>
<td>Grazing/abandonment</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>89 (9)</td>
</tr>
<tr>
<td>SC-SUT</td>
<td>Agriculture/abandonment</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>a</td>
<td>ns</td>
<td>40 (10)</td>
</tr>
<tr>
<td>FR-ERC</td>
<td>Mowing/grazing/fertilization</td>
<td>a</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>a</td>
<td>ns</td>
<td>60 (10)</td>
</tr>
<tr>
<td>FR-LAU</td>
<td>Mowing/grazing/fertilization</td>
<td>–</td>
<td>–</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>a</td>
<td>ns</td>
<td>*</td>
<td>50 (10)</td>
</tr>
<tr>
<td>NO-BER</td>
<td>Grazing</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>nd</td>
<td>nd</td>
<td>a</td>
<td>–</td>
<td>20 (5)</td>
</tr>
</tbody>
</table>

% Sites significant: 70 (10) 80 (10) 70 (10) 64 (11) 55 (11) 64 (11) 81 (11) 56 (9) 75 (8) 100 (11)

The last column gives the percentage of ecosystem/soil properties showing significant differences among treatments within a site (the number in parentheses is the number of ecosystem/soil properties available at that site), and the last row gives the percentage of sites in which a particular property varies significantly among treatments (the number in parentheses is the number of sites for which the property is available).

Abbreviations: AGBmass, maximum live standing biomass; AGTotdead, total dead material (standing dead + litter) at peak standing biomass; ANPP, above-ground net primary productivity; K decay-lab (NIRS), decay rate assessed by near-infrared spectrometry; Soil C and N are total soil carbon and nitrogen concentrations, respectively; Soil P-Olsen is plant-available phosphorus; NNI and PNI are the nitrogen and phosphorus nutrition indices, respectively. Species richness is the number of species recorded in each plot. ns, not significant; a, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. nd, no data. +, increase; –, decrease in response to decreasing land use. Hump-shaped responses are indicated by +/– or –/+. 
whether land-use effects on AGTotdead may be explained by changes in LDMC and/or in climate.

Accumulation of dead material represents the net balance between rates of production and decomposition of dead matter. Decomposition has been shown to be slower in litter produced by plants with high leaf dry matter content (Garnier et al., 2004; Kazakou et al., 2006). Our hypothesis was therefore that a reduction of management pressure would cause litter to accumulate in communities as LDMC increased due to slower decomposition (Tables 4 and 5). We further investigated whether interactions with climate were detectable across sites.

Figure 4A shows that across sites, less intensive land use led to communities with higher LDMC-aggregated values (Wald statistic = 8.45, \( P < 0.001 \)). Within sites, differences were significant for five sites, but the direction of change was consistent for ten of the 11 sites. Aggregated LDMC tended to decrease with mean annual temperature (\( P = 0.10 \)) but not with aridity or the first axis of the PCA. The increase in LDMC in response to less intensive land use was greatest at warmest sites (significant interaction; Wald statistic = 21.73, \( P < 0.001 \)), possibly as a result of a higher intensity of land use increasing the dominance by annuals at the warmest (and especially Mediterranean) sites. At sites where the differences in LDMC among treatments were largest, dead material tended to accumulate in large quantities (Fig. 4B). The quantity of dead biomass was, in some plots, up to four or five times greater than the live standing biomass, with the largest differences evident in abandoned plots (e.g. FR-HGM, CZ-OHR, SE-BAL).

As hypothesized, there was a positive relationship between aggregated-LDMC and above-ground dead biomass (Fig. 5). Five points appear as outliers in this relationship: three are from the CZ-OHR site (open circles) and two from the FR-HGM site (closed squares). In these two cases, the plots concerned are dominated by large tussock grasses with high LDMC leaves (Molinia caerulea in the Czech site, Brachypodium phoenicoides in the French site). The large accumulation of dead biomass in these plots is probably linked to the combination of high biomass production, substantial leaf turnover and particularly low rate of decomposition of the litter produced by these species. For example, we have shown that the rate of decomposition of the litter produced by \( B. \) phoenicoides was the lowest among the species screened at the Mediterranean site (Kazakou et al., 2006).

Across sites, both land use and LDMC significantly influenced total dead biomass (Wald statistics = 33.61 and 36.29, respectively, \( P < 0.001 \) in both cases). In the ‘most used’ plots, total dead biomass was significantly lower than in less disturbed plots, and plots with increasing LDMC had greater total dead biomass. A significant interaction term (\( P = 0.007 \)) indicates that the higher the mean...
LDMC at a site, the greater the effect of utilization in reducing the amount of dead matter. However, no significant effects of mean annual temperature or any other climatic variables on total dead biomass were detected.

Together, these results suggest that owing to higher litter input rates, and/or lower decomposition rates, dead matter accumulation is greater in less disturbed conditions. Community aggregated LDMC provides a good indicator of these changes. This relationship emerges partly because both LDMC and litter inputs decline with increasing land use. A further potential mechanism is that litter produced by plants with a higher LDMC decomposes more slowly (Garnier et al., 2004; Kazakou et al., 2006), leading to a longer residence time for such litter in the plots. Furthermore, it is interesting to note that, across sites, the correlation between above-ground total dead biomass and LDMC is significant for least used plots ($r = 0.86$, $P < 0.001$) but not for most used plots. This is in part explained by the much lower range of variation in LDMC across most used plots.

**DISCUSSION**

A plea for standardized protocols

Considerable efforts to standardize the measurements of plant traits have been developed in recent years (Hendry and Grime, 1993; McIntyre et al., 1999; Cornelissen et al., 2003; Knevel et al., 2005). This has resulted in a rapidly increasing global coverage of data sets on plant traits (Niinemets, 2001; Reich and Oleksyn, 2004; Wright et al., 2004; Díaz et al., 2004; Moles et al., 2005), which become available in easy and normalized format following the development of trait databases (Grime et al., 1988; Fitter and Peat, 1994; Knevel et al., 2003; Wright et al., 2004). This collective, worldwide effort is a cornerstone in the development of a more consistent view of how the environment shapes the design and functioning of plants (e.g. Grime, 2001; Westoby et al., 2002).

This is only one part of the story, however. If we are to understand quantitative relationships between plant functioning and (1) environmental factors on the one hand, and (2) ecosystem functioning on the other hand, standardization for other variables is necessary. The first issue, which constitutes one of the four themes for ‘a functional trait research program’ as proposed by McGill et al. (2006), requires a careful quantification of environmental gradients. Even if, say, a trait is theoretically responsive to a particular gradient, the detection of this response will depend on the range of variation in the environmental factors underlying this gradient (Wright et al., 2005). This is one of the key reasons why these need to be quantified with objective, comparable variables and methodologies. To address the second issue, it is necessary to select which ecosystem properties are the target of study, and to use normalized protocols for their measurements.

In the following we discuss the lessons learned through our test at the 11 VISTA sites on four main topics of broad interest to future research: the quantification of environmental gradients, the relevance of the traits selected for study and of trait aggregation, how community traits contribute to ecosystem properties, and methodologies for advanced data analysis.

**Quantification of environmental gradients**

In inter-site analyses of trait and ecosystem property responses to land-use change, environmental gradients can be grouped into three main categories: climate, disturbance and resource availability.

**Climate.** Although simple measures of climate (e.g. temperature, rainfall) may sometimes be useful to predict species/trait responses, both often respond to overall climatic conditions (integration of all aspects of climate). In addition, redundancy among variables (e.g. between mean annual temperature and solar radiation) should be avoided as much as possible when applying traditional statistical methods such as general modelling or multivariate statistics. These problems can be solved by using synthetic indices such as Thornwaite’s aridity index, simple indices of rainfall effectiveness (rainfall compared with PET), or linear projections of climatic variables on the PCA axes in Fig. 1. Calculations of these indices limited to the actual growing season at each site may better capture this ‘integrated’ aspect of the climates at the different VISTA sites. Inter-site analyses such as those presented here for leaf dry matter content (LDMC) and above-ground total dead biomass (AGToatDead) illustrate this by including climatic variables as covariates. Alternative approaches could also be developed by applying structural equation modelling (SEM) to identify direct and indirect effects of the full set of climate and other environmental variables on plant traits and ecosystem processes (see Vile et al., 2006).

**Disturbances.** Across sites, the range of disturbances encompassed in the VISTA project corresponded to the wide variety of land-use transitions currently occurring in marginal agricultural situations in Europe. Within sites, differences in disturbance regimes are large when agricultural use (grazing, mowing, crop cultivation) is compared with long-term abandonment. Differences are small when either agricultural land uses of different intensity or different stages of abandonment are compared. Our assumption is that there are general commonalities of the effects of disturbance regime on plants regardless of the disturbance type (mowing, ploughing, etc). These effects operate through the intensity (the amount of biomass loss), the return interval (time for biomass recovery) and the onset (the phenological stage affected by a disturbance impact) of disturbances, while pretreatment regimes and type of disturbances (identity of grazers in particular), which are categorical variables, operate as co-variates. Characterization of disturbance regimes by such descriptors was proposed by White and Pickett (1985) and White and Jentsch (2001). In principle, they are designed to parameterize the effects of any disturbance, either natural hazards (e.g. flooding, fire) or human land use. In practice, however, this task is not yet sufficiently solved. Although
intensity of biomass loss could be adequately assessed, e.g. by using exclosures in the case of grazing, the parameterization of return interval still represents a problem. A relatively short period of grazing, e.g. 10 days per year, results in a higher return interval than five mowing events per year. This is unsatisfactory because the different spatial scales of the two disturbances regimes are not taken into account. Land uses comprising a rotation of fallow and cultivation periods (PT-MER, SC-SUT) are also insufficiently characterized by a single figure for return interval and intensity of biomass loss.

Regardless, whether disturbance stands out as a relevant predictor of plant traits and ecosystem properties may critically depend on the variation of disturbance compared with the variation of other factors such as fertility or water availability. In addition, the impact of disturbances is context-dependent across gradients of resource availability, particularly those limiting productivity.

**Soil properties and nutrient availability.** Although standard methods to determine soil variables are available, these are not unique, and the panel of variables is large (cf. Page, 1982; Sparks, 1996). The variables available for standardized, relatively easy measurements in the field are also only proxies for ‘latent’ variables that are hypothesized to determine ecosystem dynamics (see Weiher et al., 1999), just as ‘soft’ traits are used to access ‘hard’ traits (Hodgson et al., 1999). Beyond soil standard properties such as pH and texture (from which water-holding capacity of plots was derived), the soil data collected relate to pools rather than availability of nutrients (but see below for phosphorus). Concerning C and N, routine procedures allow the determination of total contents, which are directly related to total organic matter content. There is currently no simple method to assess the amount of nitrogen a given soil can release and make available to plants. Existing methods (Bundy and Meisinger, 1994) are time consuming and difficult to run in series (soil incubations), frequently depend on the variation of disturbance compared with the variation of other factors such as fertility or water availability. In addition, the impact of disturbances is context-dependent across gradients of resource availability, particularly those limiting productivity.

**The trait toolkit and some consequences of the aggregation procedure.**

The trait list we used was derived from that proposed by Weiher et al. (1999), initially designed as a toolkit to predict vegetation responses to disturbances at the species level. It was used here at the community level, through the calculation of community-aggregated traits. One of the premises of functional plant ecology is that plant traits can be used as a general tool to analyse plant–environment relationships across biomes. From Norway to Portugal, the botanical composition (Fig. 3) is indeed very different. Which of the traits actually appeared as response traits to land-use changes across the VISTA sites?

Our results suggest that reproductive plant height responds more consistently than vegetative plant height (VPH) to land-use change (Table 4). Three reasons related to the fact that data have been collected mainly on herbaceous species may explain this result. First, it is sometimes difficult to define VPH from a morphological perspective. For example, some annual grasses produce only one rapidly heading tiller, and in some dicots a flowering stem is produced without a clearly marked vegetative stage. Second, in herbaceous plants, VPH may be highly dynamic over the growing season, as it follows vegetative growth, at least to a certain extent. This has two consequences: (1) it is highly sensitive to the date of data collection, and (2) it is very variable among replicate plants, as individuals of the same population may not necessarily be at the same developmental stage at a fixed sampling date. And third, for rosette and prostrate species, plant stature bears no particular relationship to VPH. If the objective is to assess the general stature of a plant and growth is not the target of the study, we therefore suggest that reproductive plant height (RPH), which does not suffer from the same experimental and ontogenetic problems as VPH, be used rather than vegetative height. In herbaceous species, RPH should thus be added to VPH in the trait lists proposed for functional ecology (Weiher et al., 1999; Westoby et al., 2002).

Our data also confirm that leaf traits involved in the acquisition–conservation trade-off (Chapin et al., 1993; Grime, 2001; Wright et al., 2004) such as SLA, LDMC, LNC and LPC are key to the analysis of functional responses to changes in land-use intensity (Table 4): a reduction of the grazing pressure and/or fertility and an abandonment of land tend to favour species with reduced acquisition capacities and increased nutrient conservation efficiency. Stem dry matter content can be linked to this...
On the use of community-aggregated plant traits. Community-aggregated traits were used here to detect the average functional response of vegetation on the one hand, and to relate this response to ecosystem functioning, as a test of the biomass ratio hypothesis (Grime, 1998). When the aggregated traits are calculated for variable traits, i.e. for traits differing in value according to treatment levels, then a change in their values can be caused either by variability of traits within species (e.g. a species has lower LDMC in nutrient-rich moist treatment than in nutrient-poor dry treatment), or by a change in species composition (in a nutrient-rich moist site, species composition is shifted toward a higher proportion of species with inherent low LDMC). For the test of the mass ratio hypothesis of ecosystem functioning, the change in aggregated traits is probably the most important community characteristic, regardless of whether the change was achieved through variability within species or through change in species composition. For many conservation and biodiversity studies, however, it is extremely important to know to what extent is this change caused by change in species composition. In this case, we can reverse the question, asking ‘which traits cause the species to be responsive to environmental change’ (e.g. de Bello et al., 2005). Methods are being developed to separate the effect of species composition change from the effect of trait variability within species. Species-level analysis, in contrast to community-level analyses such as those presented here, would also need to account for possible phylogenetic effects.

Components of ecosystem functioning

Our research has focused on the links between species traits and biogeochemistry, with the central hypothesis that traits involved in fundamental trade-offs such as the acquisition/conservation trade-off discussed above would scale up to components of carbon and nutrient cycles at the ecosystem level (Chapin, 1993; Lavorel and Garnier, 2002). The two ecosystem processes selected (NPP and litter decomposability) are key components in these cycles (Chapin et al., 2002). NPP represents the major input of carbon into ecosystems, and it also integrates across the system in depending on climate, nutrient supply, removal by animals, as well as the traits of the plants present. The fate of NPP is important. Accumulation of dead material has important implications for productivity and community composition (Grime, 2001; Facelli and Pickett, 1991), plays a role in regulating soil moisture (Heady, 1956), and can be a major determinant of carbon and nutrient storage and fluxes through ecosystems (Chapin, 1993). The magnitude of the (short-term) effects of dead material on plant communities can be comparable with those of competition or predation (Xiong and Nilsson, 1999), or site fertility (Sydes and Grime, 1981), and litter build-up can have major implications for species conservation (Lennartsson and Svensson, 1996; Eriksson and Ehrlén, 2001). Shifts in land use and plant species composition can influence decomposition by a number of mechanisms, including changes in the quality of the litter produced and influences on the temperature and moisture regime at the soil surface (Eviner, 2004). The proposed three-step methodology, quantifying potential decomposability in vitro, changes in the decomposition environment and their combined effects on decomposability in situ,
provides a simple and accessible method for estimating the relative decomposabilities (sensu Cornish, 1996) of litter produced at different sites. It thereby enables broad comparisons of changes in the decomposition environment and function of the soil decomposer community in response to land-use change. However, although it is becoming increasingly clear that plant species identity can have marked effects on below-ground processes (Binkley and Giardina, 1998; Evringer, 2004; Reich et al., 2005), the links between litter quality, short-term mass loss of litter and processes such as nutrient cycling and accumulation of soil organic matter are far from clear (Latter et al., 1998; Prescott, 2005). Despite its extremely widespread use as a simple, standardized and easy-to-use tool for comparative decomposition studies, the litterbag method has some drawbacks (see, for example, Heal et al., 1997). Future work needs to be aware of the limitations of focusing on the early stages of decomposition, and to develop simple, standardized measurements of other aspects of soil processes.

Data collected following the approach proposed by VISTA makes it possible to analyse direct effects of climate and land use on ecosystem properties vs. indirect effects via changes in plant traits. Indeed, land use also affects ecosystem properties directly, for example through fertilization, direct biomass removal or physical alteration of soils (e.g. trampling). This approach was illustrated above in the case of LDMC and standing dead biomass (Fig. 6), and discussed in the case of litter decomposability. For instance, our results highlighted that land use may affect standing dead biomass in two ways: (1) directly – increasing land use leads to decreasing litter accumulation, e.g. through biomass export or direct physical effects (e.g. trampling); and (2) indirectly – increasing land use leads to decreased LDMC. Because LDMC is positively correlated with litter accumulation, a negative effect of land use on litter ensues. These relationships are enhanced for sites with higher mean annual temperature both because decreases in LDMC in response to land use are greatest at warmer sites, and because decreases in litter accumulation in response to land use are greatest at warmer sites (Fig. 6). Finally, direct vs. indirect effects of land use may operate through the modification of soil properties, including land-use legacies (Fraterrigo et al., 2006; Quétier et al., 2006). In the same way that experimental approaches have been used to tease out the effects of different components of grazing on vegetation (e.g. Kohler et al., 2004), manipulative experiments that can isolate direct effects of climate and land use from indirect effects via changes in community functional composition are required to advance our understanding of the role of traits as linkages between environmental change and ecosystem properties.

Data handling and analyses

The VISTA project has collected and collated a large quantity of data of various types (climate, soil, traits) and scales (species, community, ecosystem). These data have been linked within the database to allow efficient management and flexibility in developing data analysis.

As briefly illustrated in this paper, these analyses can take a number of different forms: multiple measurements per species (as many occur in more than one site and more than one treatment) allow intra-specific variation in traits to be analysed; trait responses can be examined at different scales (within site or across sites) and levels of organization (population or community); trait responses can also be compared for consistency between the site scale and the European scale, and the effects of climate and other abiotic variables on these responses considered; traits can be examined individually (univariate) or in groups (multivariate). For instance, all leaf traits could be examined within the same analysis rather than separately, thus acknowledging that leaf traits co-vary (Díaz et al., 2004; Wright et al., 2004). The large numbers of species should make it possible to investigate phylogenetic correlations and robustly to test trait responses within life forms, as suggested by McIntyre et al. (1999).

The analytical procedures must also be appropriate and informative. They must consider the structure of the database – for example testing across sites needs linear mixed modelling rather than regression. They must also be capable of apportioning variation into different sources – allowing the examination of relationships between traits, land use, soil or climate to be done separately. Multivariate testing allows the simultaneous testing of suites of traits that might co-vary (e.g. leaf traits) or that might show little individual variation but show significant patterns when taken together. Finally, recently developed methods such as SEM will make it possible to test multivariate hypotheses regarding direct and indirect paths of causal linkages with multiple, generalized independent and dependent variables (e.g. Vile et al., 2006).

CONCLUSIONS

We have proposed a methodological framework, along with standardized protocols, to collect and analyse the large data sets that are required to test recent hypotheses regarding linkages between species and ecosystem functioning through plant traits. The application of this methodology for 11 sites representing a large diversity of climatic and land-use conditions across Europe has confirmed the relevance of plant traits associated with the
nutrient acquisition–conservation trade-off to understand the response of ecosystems to land-use change. It has highlighted other useful morphological and regeneration traits such as reproductive plant height and flowering phenology. Including environmental variables such as standardized descriptors of climate, soil and disturbance in quantitative analyses makes it possible to test hypotheses about the pathways that ultimately determine ecosystem properties directly, and indirectly through modification of plant trait representation in communities. The proposed framework and methodologies are generally applicable to analyse the impacts of global change drivers on species, communities and ecosystems.

ACKNOWLEDGEMENTS

This work formed the core of Workpackage 2 of the EU project VISTA (Vulnerability of Ecosystem Services to Land Use Change in Traditional Agricultural Landscapes) (contract no. EVK2-2001-000356). Many thanks to the various landowners who gave permission for this work and our many colleagues who helped on the project. Martial Sireix (Safir Conseil) was a constant support in the design and use of the database. We thank Sandra Díaz and Evan Weiher for helpful suggestions on the manuscript. This is a publication from the GDR 2574 ‘Utilitaires’ (CNRS, France).

LITERATURE CITED


**APPENDIX 1. NITROGEN AND PHOSPHORUS NUTRITION INDICES**

These indices were determined from above-ground live biomass data and chemical analyses conducted on this harvested biomass. When legume species were present in a sample, they were sorted by hand before chemical analysis, or their contribution to live above-ground biomass (AGBmass) was estimated in order to introduce a correction to the calculated NNI (see below). Nitrogen and phosphorus concentrations were determined from oven-dried (80 °C) ground material (0-5 mm). Total N concentration (%)N was determined either with a CN elemental analyser or with a Kjeldahl protocol following a digestion procedure, while total P (%)P was obtained following wet digestion in H2SO4/H2O2 using the ceruleomolybdenic blue method (Murphy and Riley, 1962). From these measurements, the nitrogen (NNI) and phosphorus (PNI) nutrition indices were calculated (Duru and Ducroq, 1997; Duru et al., 1997; Lemaire, 1997):

\[ NNI = \frac{\% N_{measured}}{\% N_{optimum}} \times 100; \]

\[ PNI = \frac{\% P_{measured}}{\% P_{optimum}} \times 100; \]

When %N is determined on biomass including legumes, a correction on the calculation of NNI is required (NNIc and PNIc, respectively) in order to avoid overestimations due to the ability of legumes to fix atmospheric nitrogen (Jouany et al., 2005; Cruz et al., 2006):

\[ NNIc = NNI - (0.7 \times \text{legume} \%); \]

\[ PNIc = PNI + (0.5 \times \text{legume} \%); \]

**APPENDIX 2. METHODS FOR ECOSYSTEM PROPERTIES**

**Components of primary productivity**

At least two harvests of above-ground biomass were conducted in a specific growing season at each site, to assess minimum and maximum standing biomass. This was done for all plots, each plot being considered as a treatment replicate. The details of the protocols differed, depending on type of vegetation and whether the plots were grazed or not. Permanently fenced subplots were usually used in grazed areas. In the small number of plots where woody species were present, harvests were conducted on the herbaceous layer.

An area of approx. 1 m² per plot was sampled, which appears acceptable in this type of vegetation (cf. Wiegert, 1962; Roberts et al., 1993). This area comprised smaller sample units (quadrats or circles) spread over each plot. All above-ground material (live and dead) within sampling units selected on each harvest date was collected by clipping at ground level. On this harvested biomass, live material was separated from the dead. These two fractions were then oven-dried (or freeze-dried) to constant mass at 60 °C and weighed. Above-ground net primary productivity (ANPP) was then calculated (‘Method 3’ of Scurlock et al., 2002):

\[ ANPP = \frac{(Max AGBmass) - (Min AGBmass)}{I_{Max} - I_{Min}} \]  

and specific above-ground net primary productivity (SANPP) was calculated (Garnier et al., 2004):

\[ SANPP = \log (Max AGBmass) - \log (Min AGBmass) \]  

\[ \frac{I_{Max} - I_{Min}}{I_{Max} - I_{Min}} \]
where Min. and Max. refer to the periods of minimum and maximum above-ground live standing biomass (AGBmass), respectively.

**Litter decomposition**

**Litter collection.** At the time of the major peak of natural senescence, litter of all plant parts of all vascular plant species was collected in the proportions in which it is naturally shed in the plots (Hector et al., 2000; Knops et al., 2001). Coarse woody litter (>5 mm diameter), live biomass, substantially decomposed material and seeds were excluded. In sites where there was more than one, or a long, senescence peak, several litter collections were mixed to form one ‘community litter’ (Aerts et al., 2003). Hay was chosen for the standard material. Native and standard litter were cut into 5-cm lengths, so that litter could be contained within the litter bags and each litter bag could contain a representative mixture of different plant parts and species (Dukes and Field, 2000). Litter was dried at room temperature (approx. 20°C) for 3–4 d.

A standard 1-mm mesh (Northern Mesh, Oldham, UK) was used in all sites. Litter was weighed into 2-g (± 0.1 g) portions, and placed into flat litter bags, of approx. 10 x 10 cm. Subsamples (2 g) for air dry weight to oven dry weight conversion were made. A standard procedure was followed for dealing with litter spillage.

**Field decomposition.** Incubation was initiated as soon as possible after the peak of natural senescence. Community litter was incubated in the plot from which it came. A minimum of seven litter bags per plot, harvest and litter type (community or standard) were placed on the soil surface (minimum 42 bags per plot). Where necessary, small exclosures were used to protect bags from grazing animals, and vice versa. Vegetation in exclosures was clipped regularly during the growing season to match the height of vegetation in the plot as a whole.

Three harvests were made across all sites and plots, after approx. 6, 12 and 18 months. In sites with very rapid or strongly seasonal decomposition patterns, an additional harvest, after 3 months, was performed. Standardized harvesting guidelines were followed by each partner. Any extraneous plant material, large soil animals and soil aggregates were carefully removed by hand. Ashing of litter samples was only carried out at sites where soil ingress into the bags was considered to be substantial. The litter bag contents were dried at 60°C for 3 d, and weighed.

**Decomposability in microcosms.** For each replicate of subsampled disturbance regimes per site, five litter samples of 3 g were sealed in a nylon litter bag of 1-mm mesh (Northern Mesh). Each litter sample was soaked for 24 h in 0.1 L of water and then placed on the microcosm soil (Taylor and Parkinson, 1988). The soaking water was added to keep the soluble nutrients, and the soil was moistened up to 80% of field capacity. The microcosms were kept in the dark at 22°C and watered once a week to maintain constant soil moisture. For each treatment replicate, one litter sample was removed at the end of 1, 2, 4, 6 and 8 weeks. Soil particles were taken off the litter bags and the litter samples were weighed after drying in an oven for 48 h at 55°C. The litter decomposability of each replicate was determined with the single negative exponential model (Olson, 1963):

\[
\%\text{LMR} = \frac{\text{DM}_0}{e^{-kt}}
\]

where %LMR is the percentage of litter mass remaining, DM0 is the initial dry mass and k is the litter decomposability rate over time t in days.

**Decomposability assessed by near-infrared reflectance spectroscopy (NIRS).** NIRS is a physical analysis method based on the selective absorption of near-infrared electromagnetic waves (1100–2500 nm) by the organic molecules (Birth and Hecht, 1987). NIRS was used to: (1) determine calibration equations between the initial litter spectral information and the litter decomposability ascertained in microcosms for the subsampled treatments at each site; and (2) use the calibration equations to predict the litter decomposability of the disturbance regimes that were not studied in microcosms (Gillon et al., 1999). One initial litter sample of 5 g was ground in a cyclone mill (Cyclotec Sample Mill 1093) and scanned by a NIRSystem 6500. Calibrations between initial litter spectral data and litter decomposability were calculated using the partial least squares (PLS) method with the ISI software system (Shenk and Westerhaus, 1991).