

1 **Assessing the effects of land use change on plant traits, communities and ecosystem**
2 **functioning in grasslands: a standardized methodology and lessons from an application**
3 **to 11 European sites**

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1 **Assessing the effects of land use change on plant traits, communities and ecosystem**
2 **functioning in grasslands: a standardized methodology and lessons from an application**
3 **to 11 European sites**

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16 *Running title: Methodology to assess functional effects of land use change in grasslands*

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1 **Abstract**

- 2 • *Background and Aims* A standardized methodology to assess the impacts of land use
3 changes on vegetation and ecosystem functioning is presented. It assumes that species
4 traits are central to these impacts, and is designed to be applicable in different
5 historical, climatic contexts and local settings. Preliminary results are presented to
6 show its applicability.
- 7 • *Methods* Eleven sites, representative of various types of land use changes occurring in
8 marginal agro-ecosystems across Europe and Israel, were selected. Climatic data was
9 obtained at the site level; soil data, disturbance and nutrition indices were described at
10 the plot level within sites. Sixteen traits describing plant stature, leaf characteristics,
11 and reproductive phase, were recorded on the most abundant species of each
12 treatment. These data were combined with species abundance to calculate trait values
13 weighed by the abundance of species in the communities. The ecosystem properties
14 selected were components of above-ground net primary productivity and
15 decomposition of litter.
- 16 • *Key results* The wide variety of land use systems that characterise marginal landscapes
17 across Europe was reflected by the different disturbance indices, and were also
18 reflected in soil and/or nutrient availability gradients. The trait toolkit allowed us to
19 describe adequately the functional response of vegetation to land use changes, but we
20 suggest that some traits (vegetative plant height, stem dry matter content) should be
21 left out in studies involving mainly herbaceous species. Using the example of the
22 relationship between leaf dry matter content and above-ground dead material, we
23 demonstrate how the data collected may be used to analyze direct effects of climate
24 and land use on ecosystem properties *vs.* indirect effects via changes in plant traits.

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

4

5

6 **Key words:** climate gradient, disturbance, ecosystem properties, European marginal
7 agriculture, land use change, methods, nutrient limitation, plant community, plant
8 functional traits, soil properties

9

INTRODUCTION

1
2 A conceptual framework to understand the linkages between species and ecosystem
3 functioning using plant traits was recently proposed by Chapin *et al.* (2000) and further
4 refined (Díaz and Cabido, 2001; Lavorel and Garnier, 2002). It distinguishes *functional*
5 *response traits*, which are species traits that vary consistently in response to changes in
6 environmental factors, and *functional effect traits*, which are species traits that feed back to
7 ecosystem functioning. The main hypothesis put forward by Lavorel and Garnier (2002) was
8 that traits involved in resource acquisition and use at the species level would scale-up to
9 ecosystem functioning, provided that traits are weighed by the species' contribution to the
10 community. This is known as the "biomass ratio hypothesis" (Grime, 1998). Based on a list of
11 plant traits derived from Weiher *et al.* (1999), we designed a multi-site test of this virtually
12 untested hypothesis (but see Garnier *et al.*, 2004, Quétier *et al.*, 2006), in the context of
13 changes in land use occurring and projected in marginal agro-ecosystems within Europe
14 (Klein Goldewijk, 2001; Rounsevell *et al.*, 2006). This was conducted as part of the project
15 "Vulnerability of Ecosystem Services to Land Use Change in Traditional Agricultural
16 Landscapes" (VISTA), funded by the European Union over the 2003-2005 period.

17 Our objectives were to: (i) identify changes in species traits in response to
18 modifications of land use, and (ii) use plant traits to scale-up from the functioning of species
19 to that of populations and ecosystems, using easily measurable functional traits, in a large
20 range of situations both in terms of climate (dry, wet warm, cold) and types of land use
21 changes (abandonment, reduction of grazing pressure or fertilization, etc). It was therefore a
22 prerequisite to standardize as much as possible the design and data collection across the
23 different sites. McIntyre *et al.* (1999) persuasively argued that valuable inter-site and inter-
24 study comparisons on trait response require standardization of trait and disturbance
25 measurement, but here, the questions addressed required standardization on an even wider

1 array of variables. The main aim of this paper is to describe the standardized methods
2 developed in the framework of this multi-site study designed to assess the impacts of land use
3 change on vegetation. It covers aspects pertaining to (i) the measurement of environmental
4 variables (soil and climate), plant traits at the species and whole community levels and
5 ecosystem properties; (ii) data organisation and (iii) data analyses. We present some
6 preliminary results obtained across sites to demonstrate the applicability of this methodology.
7 We then discuss methodological opportunities and constraints, as well as the scope of this
8 methodology for worldwide analyses of plant functional responses and ecosystem effects of
9 land use change.

10

11

MATERIAL AND METHODS

Overview of the experimental design

13 The study was conducted at ten sites across Europe and one site in Israel (Table 1). Within
14 each site, a number of land use regimes were identified (Table 1), considered as “treatments”
15 hereafter. These treatments can be classified in three main categories: extensification due to a
16 reduction of grazing pressure, a decrease in fertilizer input or complete land abandonment.
17 For each land use regime, a number of (in most cases) non-contiguous replicate plots were
18 selected in the landscape. The exact design was dependent on the spatial arrangement of the
19 land in each site. Within each plot, sampling areas (“subplots”) were selected to collect
20 community composition data, species traits and ecosystem properties (see below). On
21 average, there were 4.5 treatments *per* site (range: 3-8), with 4 replicate plots *per* treatment
22 (range: 2-7), giving a total of 48 treatments and 194 plots across the 11 sites (Table 1).

23

Environmental variables at site level

24

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

10

11 *Environmental variables at plot level*

12 *Soil data*. Soil samples (0-5 cm horizon) were collected at the end of the winter period with a
13 hand-auger, after the upper root mat was discarded if present. In each plot 10 to 20 cores,
14 evenly distributed on the whole plot area, were collected and bulked to make a composite
15 sample representative of the plot. Soils were crumbled by hands and dried for one week at
16 room temperature, then sieved at 2 mm before analysis. All soils samples were analysed by
17 the ‘Laboratoire d’Analyses des Sols’ of the National Institute for Agronomic Research
18 (INRA, 62000 Arras, France) using standard procedures (Afnor, 1994). The following
19 variables were measured (Table 2): pH_{water}, texture (sand, loam clay), CaCO₃, total C and N
20 concentrations; the plant available P fraction was determined by the Olsen procedure (Olsen
21 *et al.*, 1954). Water holding capacity was derived from these raw data, using a modified
22 version of the equation provided by Saxton *et al.* (1986; see
23 <http://www.bsyse.wsu.edu/saxton/>).

24 *Disturbance regimes*. The parameterisation of disturbance regimes across the VISTA sites
25 and land use types builds on disturbance descriptors developed in White and Pickett (1985),

1 Kleyer (1999), and White and Jentsch (2001). Five parameters were used to describe the
2 disturbance regime: (i) pre-treatment regime (land use category), (ii) type of disturbance (land
3 use category), (iii) return interval of disturbance (year), (iv) onset of disturbance (Julian day
4 in the year), and (v) intensity of disturbance (% biomass removed). In case of rotational
5 disturbances, each disturbance was parameterised separately and the rotation was
6 characterised by aggregating the single values.

7 *Nutrition indices.* Nitrogen (NNI) and phosphorous (PNI) indices were used to determine
8 nutrient limitation for plant growth. NNI was calculated as the ratio between the actual
9 nitrogen concentration of above ground biomass and the critical nitrogen concentration (*i.e.*
10 concentration allowing potential growth), as proposed by Lemaire and Gastal (1997). PNI,
11 which depends on nitrogen concentration of above-ground biomass, was calculated as
12 proposed by Duru and Ducrocq (1997) and Jouany *et al.* (2004). Formula and details for both
13 indices are given in Appendix 1.

14

15 *Species and traits*

16 Species selected for trait measurements were those most abundant species that collectively
17 made up at least 80% of the maximum standing live biomass of the community. This
18 threshold has been suggested to ensure a satisfactory description of community properties in
19 relation to biogeochemical cycles in ecosystems (cf. Garnier *et al.*, 2004; Pakeman and
20 Quested, 2006). Two types of data were collected on these species: (i) characteristics of the
21 taxa which were considered as invariant across sites. These are: species names, botanical
22 family, type of reproduction, life form, photosynthetic pathway, nutrient uptake strategy,
23 mycorrhizal type, Ellenberg figures for tolerance to different ecological factors. These were
24 collected from local floras and reference books, while species name followed the taxonomic
25 nomenclature given by the Euro+Med PlantBase (<http://www.euromed.org.uk/>); (ii) sixteen

1 traits - 11 quantitative, five categorical - which were measured in each of the 11 sites (Table
 2 3). These traits were selected for their known or assumed responses to the factors studied, in
 3 particular disturbance regime and/or level and nutrient availability (see numerous examples
 4 and references in *e.g.* Chapin *et al.*, 1993; Lavorel and Cramer, 1999; Grime, 2001; Lavorel
 5 and Garnier, 2002; Pausas *et al.*, 2003). For most of them, the chosen protocols and the
 6 number of replicates measured followed Cornelissen *et al.* (2003). Protocols had to be refined
 7 for three traits: clonality, pollination mode and grazing defence (categories available upon
 8 request).

9 Based on previous studies comparing intra- vs. inter-specific variability of these traits
 10 (*e.g.* Garnier *et al.*, 2001; Roche *et al.*, 2004; Fenner and Thompson, 2005, and references
 11 therein), these were grouped into either “variable” or “stable” traits *among* treatments (Table
 12 3). For *variable traits*, replicates were taken within the different subplots of a particular
 13 treatment; the trait value for this treatment was then the average over the samples taken from
 14 all the different subplots; for *stable traits*, replicates were taken in a sample of the different
 15 subplots across the different treatments; the trait value for all treatments was then the average
 16 over the samples taken from the different subplots across all treatments. Therefore, for stable
 17 traits, an average value *per species* was assigned for a site, while for variable traits, an
 18 average value *per species per treatment* was assigned.

19

20 *Species abundance and aggregated plant traits*

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

25 traits, this was done as: $trait_{agg} = \sum_{i=1}^n p_i * trait_i$ where p_i is the relative contribution of species

1 i to the community, and trait_i is the trait value of species i . For categorical traits, the relative
2 contribution of each particular attribute was calculated as the sum of relative abundances of
3 species within that attribute. In both cases, this was calculated with species that collectively
4 make up at least 80% of the maximum standing biomass of the community.

5

6 *Ecosystem properties*

7 The ecosystem properties selected for study (Table 2) are key components of carbon and
8 nitrogen cycles (Chapin *et al.*, 2002). These are: minimum and maximum live standing and
9 dead biomass (terminology after Scurlock *et al.*, 2002), above-ground net primary
10 productivity and specific above-ground net primary productivity (SANPP), rate of litter
11 decomposition, measured both in the field and in microcosms under controlled conditions in
12 the laboratory, or assessed by Near Infra-Red Spectrometry.

13 Net Primary Productivity (NPP) represents the major input of carbon and energy into
14 ecosystems. It can be considered as an integrative variable of the functioning of the whole
15 ecosystem, due to its relationships with animal biomass, secondary productivity and nutrient
16 cycling (McNaughton *et al.*, 1989). Although unbiased estimates of NPP are extremely
17 difficult to obtain (Roberts *et al.*, 1993; Scurlock *et al.*, 2002), simple methods may provide
18 fairly good indicators of the general ranking of study sites by productivity (Scurlock *et al.*,
19 2002). Among those, we chose that based on the difference between maximum and minimum
20 live above-ground biomass (ANPP) for two reasons: (i) it is by far the most frequently
21 reported productivity data in the literature (cf. Esser *et al.*, 2000), allowing us to compare our
22 own data on a very broad scale, and (ii) the determination of biomass at two different times
23 allows the computation of specific ANPP (SANPP), which expresses productivity on a
24 community biomass basis instead of a ground area basis (see Appendix 2). It was also

1 important to separate live and dead biomass, so as to take into account the specific effects of
2 dead material, as reported in the results section.

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

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

14

15 *Data base*

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

21

22 *Data analyses*

23 *Climate.* In order to better characterize the climate of the sites a Principal Component
24 Analysis was used to investigate the relationships between the sites in terms of mean annual
25 rainfall, mean annual temperature, mean annual PET, annual growing degree days, mean

1 annual solar radiation and Thornwaite's aridity index. Annual PET and solar radiation were
2 removed after a covariance analysis indicated they were highly correlated with, in particular,
3 mean annual temperature. Linear projections of the PCA axes were also used as a synthetic
4 climate variable.

5 *Botanical composition.* It was not possible to compare the botanical composition of the plots
6 at the species level due to insufficient overlap in species composition between sites. Instead it
7 was compared at the family level, representing gross scale variation in biogeographic and
8 environmental differences between sites and plots. The comparison was carried out using
9 Detrended Correspondence Analysis (DCA: Hill and Gauch, 1980) using CANOCO ver. 4
10 (ter Braak and Šmilauer, 1998) on the percentage contribution of each family to the total
11 cover of higher plants.

12 *Aggregated traits and ecosystem properties.* The overall effects of land use regime within
13 sites on the 10 continuous measured traits will be presented here (Table 4). One-way
14 ANOVAs were used to test for differences in aggregated trait values and ecosystem properties
15 across land use treatments within sites.

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

1 In the results presented in this paper we compared only the least and most intensively
2 used treatments at each site, while keeping levels as comparable as possible across sites.
3 'Most used' was taken to correspond to 'traditional use', usually associated with medium
4 fertility and high biomass removal. 'Least used' corresponded to abandonment of these
5 practices. For sites where abandonment was complete, we chose to focus on comparable
6 lengths of succession, using plots with intermediate duration of abandonment (> 10 years)
7 versus recently abandoned sites. At all sites these plots still had a strong herbaceous
8 component.

9

10

SELECTED RESULTS

11 *Climatic variables*

12 The 11 sites encompass a large amount of climatic variation. Not only do they differ in terms
13 of total rainfall, temperatures (both given in Table 1), solar radiation etc, but they also vary
14 considerably in the seasonality and periodicity of these climatic parameters. The first axis of
15 the PCA on site climatic parameters (75.7% of the variation) was largely associated with a
16 gradient of aridity coupled with site temperature and growing degree-days (Fig. 1). PCA axis
17 2 (22.1% of the variation) was overwhelmingly linked to annual rainfall, separating two
18 patterns underlying low aridity: high rainfall and mild temperatures year-round, as opposed
19 to true mountain sites with modest rainfall but low evaporation due to low temperatures
20 during rainy periods. The projections of the sites on the two axes make it possible to separate
21 the following groups of sites: (i) a group of Mediterranean sites with high temperatures and
22 aridity (GR-LAG, PT-MER and IS-KDE: site abbreviation in Table 1); FR-HGM is linked
23 with this group by high mean annual temperature but is distinguished by higher rainfall and
24 lower aridity; (ii) a group of colder sites but still with relatively low rainfall and medium
25 values of the aridity index (GE-MNP, CZ-OHR, SE-BAL); (iii) a group of sites with similar

1 temperatures to the above, but characterized by high rainfall and thus low aridity (SC-SUT,
2 FR-ERC); (iv) a group of true mountain sites (FR-LAU, NO-BER) characterized by cold
3 temperatures, a short growing season, low aridity and medium rainfall.

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

7

8 *Disturbance indices*

9 Disturbance indices of VISTA treatments reflect the wide variety of land use systems that
10 characterise marginal landscapes across Europe, as shown by the return interval and intensity
11 of disturbances for the least and most used treatments in each site (Fig. 3). The site with the
12 highest disturbance was a short-rotational crop / fallow field in Scotland (SC-SUT: short
13 return interval with the highest intensity), and the lowest was a pasture in Sweden (SE-BAL:
14 longest return interval without disturbance) abandoned for more than 60 years. Aggregated
15 return time of disturbances (Fig. 3a) and biomass removal by disturbance (Fig. 3b) show that
16 the GE-MNP, IS-KDE, PT-MER and SE-BAL sites all span a considerably large range on the
17 disturbance gradient, because they cover situations from continuing, moderately intense
18 management to advanced secondary succession. The magnitude in disturbance regimes is
19 more restricted at the NO-BER, FR-ERC, FR-LAU and FR-HGM sites. Among these, FR-
20 ERC treatments are highly disturbed, while the abandonment sequence at FR-HGM belongs
21 to the lower end of the total VISTA disturbance gradient. All other sites are intermediate with
22 respect to variation in either disturbance frequency or biomass loss.

23

24 *Vegetation*

1 The DCA (Fig. 2) showed significant turnover at the family level across all the sites and plots
2 for the first two axes of the ordination (Axes lengths of 6.58, 5.88, 2.82 and 2.55
3 respectively). These first two axes also explained a substantial part of the variation (% of total
4 inertia explained of 10.9 and 8.1 compared to 3.5 and 2.8 for axes 3 and 4 respectively). The
5 ordination clearly showed that many of the sites were relatively similar in cover at the family
6 level with a dominance of Poaceae, as well as Apiaceae, Asteraceae, Rosaceae,
7 Caryophyllaceae and Plantaginaceae. At high values on Axis 1 were the Norwegian sites
8 (NO-BER) characterised by high covers of Ericaceae and Salicaceae, as well as the late
9 successional Swedish sites with high values for the Ericaceae. Low values of Axis 1 were
10 associated with Mediterranean woodland and scrub-dominated plots, including the GR-LAG
11 and PT-MER sites dominated by Cistaceae and Fagaceae (Axis 1 scores of c. 3).

12

13 *Responses of plant traits to land use change: an overview*

14 Continuous traits (aggregated values) can be grouped into three categories, according to their
15 sensitivity to treatments across sites (Table 4): (1) traits that respond to treatments in most
16 sites: reproductive plant height, stem dry matter content, leaf phosphorus concentration and
17 flowering time, (2) traits that respond in half to two third of the sites: specific leaf area,
18 vegetative plant height, leaf nitrogen concentration and leaf dry matter content, and (3) traits
19 that respond in ca. one third of the sites: seed mass and leaf carbon concentration. The REML
20 analysis across the 11 sites highlighted strong and consistent responses across sites for all
21 traits except seed mass. Direction of change was overall consistent for all traits with a
22 significant response. Decreasing land use resulted at the community level in dominance by
23 taller plants (increased vegetative and reproductive height), plants with more conservative leaf
24 syndromes (decreased SLA, LNC and LPC, increased LDMC associated with increased
25 StDMC), and delayed flowering phenology. These responses were overall strongly consistent

1 across sites, and the few exceptions resulted from either canopy closure after woody
2 colonization (e.g. Sweden, Greece) or specific soil conditions (water-logging in Germany).

3 Multivariate analyses conducted at four individual sites identified consistent patterns
4 in variation in plant strategies. At the SC-SUT site, Pakeman and Small (2004) showed that
5 land use was a significant predictor of plant traits as abandonment of rotational cultivation led
6 to an increase in species with more conservative nutrient use, had later flowering and larger
7 seed sizes. However, species composition did not recover in the direction expected from
8 analysis of unploughed areas. Overall, these results were repeated at the French alpine site
9 (FR-LAU), where cessation of fertilization or of mowing resulted in the dominance by taller
10 plants with more conservative nutrient use and later flowering, although seed mass did not
11 vary across land use treatments (Quétier *et al.*, 2006). A path analysis conducted on the data
12 from the French Mediterranean site (FR-HGM) demonstrated that abandonment of vine
13 cultivation led to the progressive replacement of species with small stature, high rates of
14 resource capture and early flowering by species with opposite characteristics, while seed mass
15 did not show any significant trend with time after abandonment (Vile *et al.*, 2006). Finally,
16 Lepš (1999) demonstrated significant changes in species composition both as a response to
17 fertilization, and to cessation of mowing. Plant height was a very good predictor of species
18 success under fertilization, since only tall plants were able to survive in competition.

19

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

21 Maximum above-ground live (AGBmass) and dead biomass (AGTotDead) were significantly
22 different among treatments in 10 out of the 11 sites, but the effects of treatments were not
23 necessarily comparable for the two variables (Table 5). On the other hand, due to the very
24 tight correlation between maximum AGBmass and ANPP within (not shown) and across sites
25 ($r = 0.89$, $P < 0.001$, $n = 170$), the effects of treatments on these two variables were identical.

1 Treatments had significant effects on average community decomposition rate measured under
2 standard conditions, when there was no interaction with environmental conditions, in 6 out of
3 the 11 sites. This reflects a decline in litter quality (Fortunel et al. in prep), which is a major
4 determinant of *in-situ* decomposition rates (Swift *et al.* 1979, Lavelle 1993). The results of the
5 *in-situ* experiment are more complex (data not shown), however, since changes in the
6 decomposition environment within sites also play a major role.

7 There was also a very close relationship between soil C and N both within (not shown)
8 and across ($r = 0.87$, $P < 0.001$, $n = 194$) sites, with the consequence that treatment effects
9 were almost identical on the two variables (the SC-SUT site being the only exception).
10 Interestingly, nitrogen limitation as detected by the nitrogen nutrition index had little
11 concordance with information given by soil N. Phosphorus availability to plants as detected
12 by soil P-Olsen was significantly different among treatments in 9 of the sites, and except for
13 two sites (IS-KDE and FR-LAU), these differences were consistent with those found using
14 the phosphorus nutrition index.

15

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

17 One of the methodologies that can be used to assess the effects of land use change on the
18 functional properties of the vegetation through plant traits and environmental variables
19 consists in successive sets of general modelling analyses. We present here the case of leaf dry
20 matter content and above-ground total dead biomass as an illustration.. Our approach
21 consisted in first identifying the effects of climate and land use factors on LDMC, and then
22 testing whether land use effects on AGTotdead may be explained by changes in LDMC
23 and/or in climate.

24 Accumulation of dead material represents the net balance between rates of production and
25 decomposition of dead matter. Decomposition has been shown to be slower in litter produced

1 by plants with high leaf dry matter content (Garnier *et al.*, 2004; Kazakou *et al.*, 2006). Our
2 hypothesis was therefore that a reduction of management pressure would cause litter to
3 accumulate in communities as LDMC increased due to slower decomposition (Tables 4 and
4 5). We further asked whether interactions with climate were detectable across sites.

5 Fig. 4a shows that across sites, less intensive land-use led to communities with higher
6 LDMC-aggregated values (Wald statistic = 8.45, $P < 0.001$). Within sites, differences were
7 significant for 5 sites, but the direction of change was consistent for 10 of the 11 sites.
8 Aggregated LDMC tended to decrease with mean annual temperature ($P = 0.10$) but not with
9 aridity or the first axis of PCA. The increase in LDMC in response to less intensive land use
10 was greatest at warmest sites (significant interaction; Wald statistic = 21.73, $P < 0.001$),
11 possibly as a result of a higher intensity of land use increasing the dominance by annuals at
12 the warmest (and especially Mediterranean) sites. In sites where the differences in LDMC
13 among treatments were largest, dead material tended to accumulate in large quantities (Fig.
14 4b). The quantity of dead biomass was, in some plots, up to 4 or 5 times greater than the live
15 standing biomass, with the largest differences evident in abandoned plots (*e.g.* FR-HGM, CZ-
16 OHR, SE-BAL). As hypothesised, there was a positive relationship between aggregated-
17 LDMC and above-ground dead biomass (Fig. 5). Five points appear as outliers in this
18 relationship: three are from the CZ-OHR (white circles) and two from the FR-HGM site
19 (black squares). In these two cases, the plots concerned are dominated by large tussock
20 grasses with high LDMC leaves (*Molinia caerulea* in the Czech site, *Brachypodium*
21 *phoenicoides* in the French site). The large accumulation of dead biomass in these plots is
22 probably linked to the combination of high biomass production, substantial leaf turnover, and
23 particularly slow decomposability of the litter produced by these species. For example, we
24 have shown that the rate of decomposition of the litter produced by *B. phoenicoides* was the
25 slowest among the species screened at the Mediterranean site (Kazakou *et al.*, 2006).

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

8 Together, these results suggest that due to higher litter input rates, and /or slower
9 decomposition rates, dead matter accumulation is greater in less disturbed conditions.
10 Community aggregated LDMC provides a good indicator of these changes. This relationship
11 emerges partly because both LDMC and litter inputs decline with increasing land use. A
12 further potential mechanism is that litter produced by plants with a higher LDMC decomposes
13 more slowly (Garnier *et al.*, 2004; Kazakou *et al.*, 2006), leading to a longer residence time
14 for such litter in the plots. Further, it is interesting to note that, across sites, the correlation
15 between above-ground total dead biomass and LDMC is significant for least used plots ($r =$
16 0.86 , $P < 0.001$) but not for most used plots. This is in part explained by the much lower
17 range of variation in LDMC across most used plots.

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DISCUSSION

A plea for standardized protocols

21 Considerable efforts to standardize the measurements of plant traits have been developed in
22 recent years (Hendry and Grime, 1993; McIntyre *et al.*, 1999; Cornelissen *et al.*, 2003;
23 Knevel *et al.*, 2005). This has resulted in a rapidly increasing global coverage of data sets on
24 plant traits (Niinemets, 2001; Reich and Oleksyn, 2004; Wright *et al.*, 2004; Díaz *et al.*, 2004;
25 Moles *et al.*, 2005), which become available in easy and normalized format following the

1 development of trait data bases (Grime *et al.*, 1988; Fitter and Peat, 1994; Knevel *et al.*, 2003;
2 Wright *et al.*, 2004). This collective, worldwide effort is a corner stone in the development of
3 a more consistent view of how the environment shapes the design and functioning of plants
4 (*e.g.* Grime, 2001; Westoby *et al.*, 2002).

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

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

21

22 *Quantification of environmental gradients*

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

1 *Climate.* Although simple measures of climate (*e.g.* temperature, rainfall) may sometimes be
2 useful to predict species/trait responses, both often respond to overall climatic conditions
3 (integration of all aspects of climate). In addition, redundancy among variables (*e.g.* between
4 mean annual temperature and solar radiation) should be avoided as much as possible when
5 applying traditional statistical methods such as general modelling or multivariate statistics.
6 These problems can be solved by using synthetic indices such as the aridity index of
7 Thornwaite, simple indices of rainfall effectiveness (rainfall compared to PET), or linear
8 projections of climatic variables on the PCA axes in Fig. 1. Calculations of these indices
9 limited to the actual growing season at each site may better capture this “integrated” aspect of
10 the climates at the different VISTA sites. Inter-site analyses such as the ones presented here
11 for Leaf Dry Matter Content (LDMC) and Aboveground Total Dead biomass (AGTotDead)
12 illustrate this by including climatic variables as covariates. Alternative approaches could also
13 be developed by applying Structural Equation Modelling (SEM) to identify direct and indirect
14 effects of the full set of climate and other environmental variables on plant traits and
15 ecosystem processes (see Vile *et al.*, 2006).

16 *Disturbances.* Across sites, the range of disturbances encompassed in the VISTA project
17 corresponded to the wide variety of land use transitions currently occurring in marginal
18 agricultural situations in Europe. Within sites, differences in disturbance regimes are large
19 when agricultural use (grazing, mowing, crop cultivation) is compared to long-term
20 abandonment. Differences are small when either agricultural land uses of different intensity
21 are compared or different stages of abandonment. Our assumption is that there are general
22 commonalities of the effects of disturbance regime on plants regardless of the disturbance
23 type (*e.g.* mowing, ploughing, etc). These effects operate through the intensity (the amount of
24 biomass loss), the return interval (time for biomass recovery), and the onset (the phenological
25 stage affected by a disturbance impact) of disturbances, while pre-treatment regimes and type

1 of disturbances (identity of grazers in particular) which are categorical variables, operate as
2 co-variates. Characterisation of disturbance regimes by such descriptors was proposed by
3 White & Pickett (1985) and White & Jentsch (2001). In principal, they are designed to
4 parameterise the effects of any disturbance, either natural hazards (e.g. flooding, fire) or
5 human land use. In practice however, this task is not yet sufficiently solved. While intensity
6 of biomass loss could be adequately assessed, e.g. by using exclosures in case of grazing, the
7 parameterisation of return interval still represents a problem. A relatively short period of
8 grazing, e.g. 10 days per year, results in a higher return interval than 5 mowing events per
9 year. This is unsatisfactory because the different spatial scales of the two disturbances
10 regimes are not taken into account. Land uses comprising a rotation of fallow and cultivation
11 periods (PT-MER, SC-SUT) are also insufficiently characterised by a single figure for return
12 interval and intensity of biomass loss.

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

18 *Soil properties and nutrient availability.* Although standard methods to determine soil
19 variables are available, these are not unique, and the panel of variables is large (cf. Page,
20 1982; Sparks, 1996). The variables available for standardized, relatively easy measurements
21 in the field are also only proxies for ‘latent’ variables that are hypothesized to determine
22 ecosystem dynamics (see Weiher et al., 2004), just as ‘soft’ traits are used to access ‘hard’
23 traits (Hodgson et al., 1999). Beyond soil standard properties such as pH and texture (from
24 which water holding capacity of plots was derived), the soil data collected relates to pools
25 rather than availability of nutrients (but see below for phosphorus). Concerning C and N,

1 routine procedures allow the determination of total contents which are directly related to total
2 organic matter content. There is currently no simple method to assess the amount of nitrogen
3 a given soil can release and make available to plants. Existing methods (Bundy and
4 Meisinger, 1994) are time consuming and difficult to run in series (soil incubations), making
5 the assessment of nitrogen availability for a large number of samples problematic. Therefore,
6 and since total soil N content is not informative of N availability, nitrogen nutrition indices
7 (NNI) have been developed (Lemaire and Gastal, 1997). NNI is based on plant N
8 concentration determination and gives directly the vegetation nutrient status in relation to the
9 degree of growth. The NNI has been successfully applied to diagnose N nutritional status on
10 mixed crops (Cruz and Soussana, 1997) as well as native grassland (Duru *et al.*, 1997). When
11 applied to the VISTA experimental sites, there was a higher correlation between ANPP and
12 NNI across sites ($r = 0.79$, $P < 0.001$, $n = 94$) than between ANPP and total soil N ($r = 0.64$, P
13 < 0.001 , $n = 94$), and NNI explained a greater part of the variance than any of the climatic
14 indices.

15 As for phosphorus, although total soil P content does not inform on the amount of
16 available P released from soil, several P availability indices can be obtained routinely (Kuo,
17 1996). Amongst those, Olsen P (Olsen *et al.*, 1954) has been chosen here, since the method
18 gives better characterization than other chemical extraction over a large range of pH, from
19 calcareous to slightly acidic soils (Morel *et al.*, 2000). It is also widely used for all soils types
20 in a large number of countries (Sibbesen and Sharpley, 1997). To complement these data, we
21 also calculated a phosphorus nutrition index (PNI), based on simultaneous measurements of N
22 and P concentrations of the vegetation (Appendix 1). PNI, which has been developed to assess
23 the phosphorus nutrition status in grasslands, was positively correlated with Olsen P values
24 within sites (data not shown) as already found by Jouany *et al.* (2002). However, no relation
25 exists between these two variables at the inter-site level.

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The trait toolkit and some consequences of the aggregation procedure

The trait toolkit. 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 relations across biomes. From Norway to Portugal, the botanical composition (Fig. 2) 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 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, since it follows vegetative growth, at least to a certain extent. This has two consequences: (i) it is highly sensitive to the date of data collection, and (ii) it is very variable among replicate plants, since 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, 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).

1 Our data also confirm that leaf traits involved in the acquisition-conservation trade-off
2 (Chapin *et al.*, 1993; Grime, 2001; Wright *et al.*, 2004) such as SLA, LDMC, LNC and LPC
3 are key to the analysis of functional responses to changes in land use intensity (Table 4): a
4 reduction of the grazing pressure and/or fertility and an abandonment of land tend to favour
5 species with reduced acquisition capacities and increased nutrient conservation efficiency.
6 Stem dry matter content can be linked to this group of traits, and appears to be quite
7 responsive as well. However, this trait is not always easy to measure, in particular because it
8 is not easy to assess whether rehydration of the stem *prior* to measurement is complete. Even
9 though StDMC - a proxy of stem tissue density - plays an important role for the structure and
10 functioning of woody species (see Cornelissen *et al.* 2003 and references therein), we suggest
11 that its efficacy and standardized measurement have not been sufficiently established to
12 recommend its standard use in studies involving mainly herbaceous species.

13 Onset of flowering (OFL) was one of the traits most sensitive to land use changes (Table
14 4), although this trait is not commonly documented, e.g. in grazing studies (see Díaz *et al.* *in*
15 *press*). Reduced grazing, lower fertility and land abandonment promoted delayed flowering,
16 consistent with the idea that early flowering reflects disturbance avoidance (Weiher *et al.*,
17 1999; Pakeman, 2004). More generally these variations in OFL can be interpreted as a change
18 from ruderal to competitive strategies (cf. Grime, 2001; Kahmen and Poschold, 2004;
19 Pakeman, 2004), in line with the fact that large plants tend to flower late. By contrast, seed
20 mass, a trait that authors tend to focus on more frequently, was responsive to treatments in
21 less than half of the sites (Table 4). This does not support the theoretical expectation that
22 small seeded species, with increased probabilities of dispersal, should be favoured in
23 disturbed habitats (Grime, 2001; Weiher *et al.*, 1999 and references therein). It may be
24 explained by the fact that seed mass varied by up to 5 orders of magnitude within individual
25 treatments, so that any patterns of response were obscured by natural variance.

1 Against these general trends, trait responses were variable across sites and need to be
2 related to the ranges of within-site variations of (i) disturbances and fertility (see above), and
3 (ii) inter *vs.* intra-treatment variability in trait values.

4 Further differences across sites in trait responses will be analyzed using climate variables
5 as covariates, as done here for the LDMC example (see also Díaz *et al.*, in press). Analyses
6 would also need to include the contribution of changes in life-form frequency as a covariate
7 (cf. McIntyre *et al.*, 1999), *e.g.* changes in grasses *vs.* forbs, and increases in the
8 representation of annuals in more disturbed treatments.

9 Apart from the restrictions discussed above, our trait list finally appears to capture
10 adequately the functional response of herbaceous-dominated vegetation to land use changes,
11 even though they differ in nature and intensity across sites. Although we expect the core of
12 the trait list to be generic, it would need to be refined for application to woody vegetation, as
13 relevant traits to response to land use and other disturbances can be life-form specific (Mc
14 Intyre *et al.*, 1999).

15 *On the use of community-aggregated plant traits.* Community-aggregated traits were used
16 here to detect the average functional response of vegetation on the one hand, and to relate this
17 response to ecosystem functioning, as a test of the biomass ratio hypothesis (Grime, 1998).
18 When the aggregated traits are calculated for variable traits, *i.e.* for traits differing in value
19 according to treatment levels, then a change in their values can be caused either by variability
20 of traits within species (*e.g.* a species has lower LDMC in nutrient rich moist treatment than
21 in nutrient poor dry treatment), or by a change in species composition (in nutrient rich moist
22 site, species composition is shifted toward higher proportion of species with inherent low
23 LDMC). For the test of the mass ratio hypothesis of ecosystem functioning, the change in
24 aggregated traits is probably the most important community characteristic, regardless whether
25 the change was achieved through variability within species or through change in species

1 composition. For many conservation and biodiversity studies, however, it is extremely
2 important to know to what extent is this change caused by change in species composition. In
3 this case, we can reverse the question, asking “which traits cause the species to be responsive
4 to environmental change” (e.g. de Bello *et al.*, 2005). Methods are being developed to
5 separate the effect of species composition change from the effect of trait variability within
6 species. Species-level analysis, in contrast to community-level analyses such as the ones
7 presented here, would also need to account for possible phylogenetic effects.

8

9 *Components of ecosystem functioning*

10 Our research has focused on the links between species traits and biogeochemistry, with the
11 central hypothesis that traits involved in fundamental trade-offs such as the
12 acquisition/conservation trade-off discussed above would scale up to components of carbon
13 and nutrient cycles at the ecosystem level (Chapin, 1993; Lavorel and Garnier, 2002). The
14 two ecosystem processes selected (Net Primary Productivity NPP and litter decomposability)
15 are key components in these cycles (Chapin *et al.*, 2002). NPP represents the major input of
16 carbon into ecosystems, and it also integrates the across the system in depending on climate,
17 nutrient supply, removal by animals, as well as the traits of the plants present.

18 The fate of NPP is important. Accumulation of dead material has important
19 implications for productivity and community composition (Grime, 1979; Facelli and Pickett,
20 1991), plays a role in regulating soil moisture (Heady, 1956), and can be a major determinant
21 of carbon and nutrient storage and fluxes through ecosystems (Chapin, 1993). The magnitude
22 of the (short term) effects of dead material on plant communities can be comparable with
23 those of competition or predation (Xiong and Nilsson, 1999), or site fertility (Sydes and
24 Grime, 1981), and litter build up can have major implications for species conservation
25 (Lennartsson and Svensson, 1996; Eriksson and Ehrlén, 2001). Shifts in land use and plant

1 species composition can influence decomposition by a number of mechanisms, including
2 changes in the quality of the litter produced and influences on the temperature and moisture
3 regime at the soil surface (Eviner, 2004). The proposed three-step methodology, quantifying
4 potential decomposability *in vitro*, changes in the decomposition environment, and their
5 combined effects on decomposability *in-situ*, provides a simple and accessible method for
6 estimating the relative decomposabilities (sensu Cornelissen, 1996) of litter produced at
7 different sites. It enables thereby broad comparisons of changes in the decomposition
8 environment and function of the soil decomposer community in response to land use change.
9 However, although it is becoming increasingly clear that plant species identity can have
10 marked effects on below ground processes (Binkley and Giardina 1998, Reich *et al.* 2005,
11 Eviner, 2004), the links between litter quality, short-term mass loss of litter and processes
12 such as nutrient cycling and accumulation of soil organic matter are far from clear (Latter *et*
13 *al.*, 1998; Prescott, 2005). Despite its extremely widespread use as a simple, standardized and
14 easy to use tool for comparative decomposition studies, the litterbag method has some
15 drawbacks (see *e.g.* Heal *et al.*, 1997). Future work needs to be aware of the limitations of
16 focussing on the early stages of decomposition, and develop simple, standardized
17 measurements of other aspects of soil processes.

18 Data collected following the approach proposed by VISTA makes it possible to analyze direct
19 effects of climate and land use on ecosystem properties *vs.* indirect effects via changes in
20 plant traits. Indeed, land use also affects ecosystem properties directly, for example through
21 fertilization, direct biomass removal, or physical alteration of soils (*e.g.* trampling). This
22 approach was illustrated above in the case of LDMC and standing dead biomass (Fig. 6), and
23 discussed in the case of litter decomposability. For instance, our results highlighted that land
24 use may affect standing dead biomass in two ways. 1) Directly: increasing land use leads to
25 decreasing litter accumulation, *e.g.* through biomass export or direct physical effects (*e.g.*

1 trampling); 2) Indirectly: increasing land use leads to decreased LDMC. Because LDMC is
2 positively correlated with litter accumulation, a negative effect of land use on litter ensues.
3 These relationships are enhanced for sites with higher mean annual temperature both because
4 decreases in LDMC in response to land use are greatest at warmer sites, and decreases in litter
5 accumulation in response to land use are greatest at warmer sites (Fig. 6). Finally, direct vs
6 indirect effects of land use may operate through the modification of soil properties, including
7 land use legacies (Quétier et al. 2006, Fraterrigo et al. 2006). In the same way that
8 experimental approaches have been used to tease out the effects of different components of
9 grazing on vegetation (e.g. Kohler et al. 2004), manipulative experiments that can isolate
10 direct effects of climate and land use from indirect effects via changes in community
11 functional composition are required to advance our understanding of the role of traits as
12 linkages between environmental change and ecosystem properties.

13

14 *Data handling and analyses*

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

18 As briefly illustrated in this paper, these analyses can take a number of different
19 forms: multiple measurements per species (as many occur in more than one site and more
20 than one treatment) allow intra-specific variation in traits to be analysed; trait responses can
21 be examined at different scales (within site or across sites) and levels of organization
22 (population or community); trait responses can also be compared for consistency between the
23 site scale and the European scale, and the effects of climate and other abiotic variables on
24 these responses considered; traits can be examined individually (univariate) or in groups
25 (multivariate). For instance all leaf traits could be examined within the same analysis rather

1 than separately, thus acknowledging that leaf traits co-vary (Díaz *et al.*, 2004; Wright *et al.*,
2 2004). The large numbers of species should make it possible to investigate phylogenetic
3 correlations and to robustly test trait responses within life forms, as suggested by McIntyre *et*
4 *al.* (1999).

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

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CONCLUSION

17 We have proposed a methodological framework, along with standardized protocols, to collect
18 and analyze the large data sets that are required to test recent hypotheses about linkages
19 between species and ecosystem functioning through plant traits. The application of this
20 methodology for 11 sites representing a large diversity of climatic and land use conditions
21 across Europe has confirmed the relevance of plant traits associated with the nutrient
22 acquisition-conservation trade-off to understand the response of ecosystems to land use
23 change. It has highlighted other useful morphological and regeneration traits such as
24 reproductive plant height and flowering phenology. Including environmental variables such as
25 standardized descriptors of climate, soil and disturbance in quantitative analyses makes it

1 possible to test hypotheses about the pathways that ultimately determine ecosystem properties
2 directly, and indirectly through modification of plant trait representation in communities. The
3 proposed framework and methodologies are generally applicable to analyse the impacts of
4 global change drivers on species, communities and ecosystems.

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CAPTIONS TO FIGURES

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FIG. 1. Principal component analysis on climatic data: mean annual rainfall (rainfall), mean annual temperature (temp), annual growing degree days (GDD), and an aridity index for each site. Annual PET and solar radiation were removed after a covariance analysis indicated they were highly correlated with, in particular, mean annual temperature.

FIG. 2. Detrended correspondence analysis of relative abundance of higher plant families for each recorded plot. Only families with a weight of > 2 % of the maximum family's weight are shown as the first eight letters of their full name.

FIG. 3. (a) Return interval (Log scale) and (b) intensity (expressed as a percentage of maximum standing biomass) of disturbance for the least (black columns) and most (grey columns) intensively used treatments in each site. In (a), a value of 0 is for a return interval of 1 year (*e.g.* both treatments in FR-LAU), while in (b) a value of 0 corresponds to a lack of biomass removal (*e.g.* both treatments in FR-HGM).

FIG. 4. (a) Community-aggregated values of leaf dry matter content, and (b) above-ground total dead material (which includes standing dead biomass and litter fallen on the ground) at time of peak biomass for the least (black columns) and most (grey columns) intensively used treatments in each site. Significance of tests by one-way ANOVAs within sites is indicated: ns: not significant, $P > 0.10$; a, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

1 FIG. 5. Relationship between community-aggregated leaf dry matter content and above-
2 ground dead biomass at time of maximum standing biomass across 10 sites of the VISTA
3 project, using the least/most used treatments in each site. r are Pearson correlation coefficients
4 for all data (top value) and when the five points with AGTotdead values higher than 600 g m^{-2}
5 were removed (in parentheses); n is the number of data points (***: both relationships are
6 significant at $P < 0.001$). Different symbols are for different sites: white squares: IS-KDE;
7 white triangles, point up: PT-MER; grey squares: GR-LAG; black squares: FR-HGM; black
8 circles: GE-MNP; white circles: CZ-OHR; grey circles: SE-BAL; grey triangles, point down:
9 SC-SUT; black triangles, point up: FR-ERC; white triangles, point down: FR-LAU.

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11 FIG. 6. Articulating direct and indirect effects of environmental factors and land use on plant
12 traits and ecosystem properties. The relationships are illustrated here based on the results
13 obtained for community aggregated LDMC and standing litter (AGTotdead). The arrows
14 represent significant statistical relationships, with +/- signs indicating the direction of effects.

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APPENDIX 1: NITROGEN AND PHOSPHORUS NUTRITION INDICES

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3 These indices were determined from above-ground live biomass data and chemical analyses
4 conducted on this harvested biomass. When legumes species were present in a sample, they
5 were sorted by hand before chemical analysis, or their contribution to live aboveground
6 biomass (AGBmass) estimated in order to introduce a correction to the calculated NNI (see
7 below). Nitrogen and phosphorus concentrations were determined on oven dried (80°C)
8 ground material (0.5 mm). Total N concentration (%N) was determined either with a CN
9 elemental analyser or with a Kjeldahl protocol following a digestion procedure, while total P
10 (%P) was obtained following wet digestion in H₂SO₄-H₂O₂ using ceruleomolybdic blue
11 method (Murphy and Riley, 1962). From these measurements, the nitrogen (NNI) and
12 phosphorus (PNI) nutrition indices were calculated (Lemaire and Gastal, 1997; Duru and
13 Ducrocq, 1997; Duru *et al.*, 1997):

$$14 \quad \text{NNI} = \%N_{\text{measured}} / \%N_{\text{optimum}} * 100; \text{ with } \%N_{\text{optimum}} = 4.8\% (\text{AGBmass})^{-0.32} \quad (\text{A1.1})$$

$$15 \quad \text{PNI} = (\%P_{\text{measured}} / \%P_{\text{optimum}}) * 100; \text{ with } \%P_{\text{optimum}} = 0.15 + 0.065 \%N_{\text{measured}} \quad (\text{A1.2})$$

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

$$19 \quad \text{NNI}_c = \text{NNI} - (0.7 * \text{legume } \%) \quad (\text{A1.3})$$

$$20 \quad \text{PNI}_c = \text{PNI} + (0.5 * \text{legume } \%) \quad (\text{A1.4})$$

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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 in each site, to assess minimum and maximum standing biomass. This was done for all plots, each plot being considered as a treatment replicate (cf Fig. 1). 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 *ca.* 1 m² per plot was sampled, which appears acceptable in this type of vegetation (cf. Wiegert, 1962; Roberts *et al.*, 1993). This area was composed from 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):

$$ANPP = \frac{(Max. AGBmass) - (Min. AGBmass)}{t_{Max} - t_{Min}} \quad (A2.1)$$

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

$$SANPP = \frac{\log(Max. AGBmass) - \log(Min. AGBmass)}{t_{Max} - t_{Min}} \quad (A2.2)$$

where Min and Max refer to the periods of minimum and maximum above-ground live standing biomass (AGBmass), respectively.

1 *Litter decomposition*

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

11 A standard 1 mm mesh (Normesh, Oldham, UK) was used in all sites. Litter was weighed into
12 2 g (+/- 0.1 g) portions, and placed into flat litterbags, of c. 10 cm by 10 cm. Subsamples (2 g)
13 for air dry weight to oven dry weight conversion were made. A standard procedure was
14 followed for dealing with litter spillage.

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

21 Three harvests were made across all sites and plots, after ca. 6, 12 and 18 months. In sites
22 with very rapid or strongly seasonal decomposition patterns, an additional harvest, after 3
23 months, was performed. Standardised harvesting guidelines were followed by each partner.
24 Any extraneous plant material, large soil animals and soil aggregates were carefully removed
25 by hand. Ashing of litter samples was only carried out at sites where soil ingress into the bags

1 was considered to be substantial. The litterbag contents were dried at 60 ° C for three days,
2 and weighed.

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

$$13 \quad \%LMR = DM_0 * e^{-kt} \quad (A2.3)$$

14 where %LMR is the percentage of Litter Mass Remaining, DM_0 is the initial Dry Mass and k
15 is the litter decomposability rate over time t in days.

16 *Decomposability assessed by Near Infrared Reflectance Spectroscopy (NIRS):* NIRS is a
17 physical analysis method based on the selective absorption of near infrared electromagnetic
18 waves (1100 - 2500 nm) by the organic molecules (Birth and Hecht, 1987). NIRS was used
19 to: (i) determine calibration equations between the initial litter spectral information and the
20 litter decomposability ascertained in microcosms for the subsampled treatments at each site;
21 and (ii) use the calibration equations to predict the litter decomposability of the disturbance
22 regimes that were not studied in microcosms (Gillon *et al.*, 1999). One initial litter sample of
23 5 g was ground in a cyclone mill (Cyclotec Sample Mill 1093) and scanned by a NIRSystem
24 6500. Calibrations between initial litter spectral data and litter decomposability were

- 1 calculated using the partial least square (PLS) method with the ISI software system (Shenk
- 2 and Westerhaus, 1991).

TABLE 1. Main geographical, topo-climate characteristics, land use change and primary disturbance of the 11 sites of the VISTA project. Temp. and rainfall are mean annual values, usually taken over 30-year periods. The numbers of treatments (treats) and plots (cf. Fig. 1) in each site are given in the last column.

Country	Name (abbreviation)	Coordinates	Altitude range (m)	Type of climate	Temp. (°C)	Rainfall (mm)	Land use change	Primary disturbance	No. of treats/plots
Israël	Karei Deshe (IS-KDE)	32°55 N 32°35 E	150	Mediterranean semi-arid	19.6	572	Abandonment/ extensification	Grazing	4/8
Portugal	Mértola/ Castro Verde (PT-MER)	37°40 N 8° 00 W	100-150	Mediterranean	16.6	538	Extensification/ abandonment	Grazing/mowing	4/12
Greece	Lagadas (GR-LAG)	40°47 N 23°12 E	450-550	Mediterranean semi-arid	12.1	586	Extensification/ abandonment	Grazing/ploughing/ cutting	4/16
France	Hautes Garrigues du Montpelliérais (FR-HGM)	43°51 N 3°56 E	100-160	Mediterranean sub-humid	13.2	994	Abandonment	Perennial crops	3/12
Germany	Müritz National Park (GE-MNP)	53°27 N 12°44 E	65	Temperate	8.7	639	Extensification/ abandonment	Grazing	8/56
Czech Republic	Ohrazeni (CZ-OHR)	48°57 N 14°36 E	510	Central-european temperate	8.2	583	Experimental regimes	Mowing	4/12
Sweden	South East Baltic Sea (SE-BAL)	58°50 N 17°24 N	0-50	Cold temperate	6.0	551	Abandonment/ extensification	Crops/grazing/ mowing	4/20
United Kingdom (Scotland)	South Uist (Staoinebrig) (SC-SUT)	57°16 N 7°24 W	0-15	Oceanic	8.4	1275	Abandonment	Rotational agriculture	3/9
France	Ercé (FR-ERC)	42°50 N 1°17 E	600-1000	Mountain humid	10.0	1079	Extensification	Grazing/mowing	6/18
France	Col du Lautaret (FR-LAU)	45°02 N 6°21 E	1900-2100	Sub-alpine	3.0	902	Extensification	Mowing/grazing/ ploughing	5/15
Norway	Båttjønndalen/ Berghøgda (NO-BER)	62°42 N 11°05 E	800-900	Alpine	0.6	750	Grazing int. /Intens/ Aband	Grazing	4/16

TABLE 2. List of community, soil and ecosystem variables measured at the plot level, with their abbreviation and unit when relevant. For ecosystem biomass components, the terminology is from Scurlock *et al.* (2002)

	Abbreviation	Unit
<i>Community</i>		
Species richness	<i>S</i>	-
Species abundance	<i>x</i>	-
Species evenness	<i>E</i>	-
Shannon-Weaver index	<i>H</i>	-
<i>Soil properties and nutrition indices</i>		
Proportion of clay	clay	%
Proportion of loam	loam	%
Proportion of sand	sand	%
pH _{water}	pH	-
Carbonate concentration	Soil CaCO ₃	mg g ⁻¹
Carbon concentration	Soil C	mg g ⁻¹
Nitrogen concentration	Soil N	mg g ⁻¹
Plant available phosphorus	Olsen P	mg g ⁻¹
Water Holding Capacity	WHC	cm ³ cm ⁻³
Nitrogen nutrition index	NNI	%
Phosphorus nutrition index	PNI	%
<i>Ecosystem</i>		
Above-ground live biomass	AGBmass	g m ⁻²
Above-ground total dead matter (standing dead + litter)	AGTotdead	g m ⁻²
Above-ground net primary productivity	ANPP	g m ⁻² d ⁻¹
Specific above-ground net primary productivity	SANPP	g kg ⁻¹ d ⁻¹
Litter decay rate of native vegetation in the field	K _{nat-field}	g kg ⁻¹ d ⁻¹
Litter decay rate of standard material in the field	K _{std-field}	g kg ⁻¹ d ⁻¹
Litter decay rate of native vegetation assessed under standard conditions (microcosms and/or near infra-red spectroscopy)	K _{nat-lab}	g kg ⁻¹ d ⁻¹
Litter decay rate of standard material assessed under standard conditions (microcosms and/or near infra-red spectroscopy)	K _{std-lab}	g kg ⁻¹ d ⁻¹

TABLE 3. List of traits retained, their abbreviation and unit, if not categorical: six of them are known to be variable within species according to abiotic and/or biotic conditions, while the remaining 10 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.

	Abbreviation	Unit
<i>Variable traits</i>		
Clonality	-	Categorical
Vegetative plant height	VPH	cm
Reproductive plant height	RPH	cm
Leaf nitrogen concentration	LNC	mg g ⁻¹
Leaf phosphorus concentration	LPC	mg g ⁻¹
Onset of flowering	OFL	day of year
<i>Stable traits*</i>		
Life history	-	Categorical
Plant height from floras	FPH	cm
Specific leaf area	SLA	m ² kg ⁻¹
Leaf dry matter content	LDMC	mg g ⁻¹
Stem dry matter content	StDMC	mg g ⁻¹
Leaf carbon concentration	LCC	mg g ⁻¹
Seed mass	SM	mg
Dispersal mode	-	Categorical
Pollination mode	-	Categorical
Grazing defences	-	Categorical

* in some sites, all traits were considered as “variable”, and were therefore measured in all treatments (see text for details)

TABLE 4. Summary of significant differences in aggregated means of the 10 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). The last column gives the percentage of traits showing significant differences among treatments within a site (the number between brackets shows the number of traits available at that site), and the last line gives the percentage of sites in which a particular trait varies significantly among treatments (the number between brackets shows the number of sites for which the trait is available). The final line presents 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 -/+.

<i>Site</i>	<i>Disturbance/resource treatments</i>	<i>VPH</i>	<i>RPH</i>	<i>Flower</i>	<i>Seed mass</i>	<i>SLA</i>	<i>LDMC</i>	<i>LNC</i>	<i>LCC</i>	<i>LPC</i>	<i>StDMC</i>	<i>% traits signif.</i>
IS-KDE	Grazing/Abandonment	<i>nd</i>	***	ns	ns	ns	ns	*	*	ns	<i>nd</i>	38 (8)
PT-MER	Agric./Grazing/Aband.	***	<i>nd</i>	***	*	***	***	ns	***	***	<i>nd</i>	88 (8)
GR-LAG	Grazing/Abandonment	ns	**	***	ns	**	ns	*	ns	***	*	60 (10)
FR-HGM	Abandonment	ns	***	**	ns	***	**	***	ns	*	***	70 (10)
GE-MNP	Grazing/Aband./Water	**	***	***	**	***	***	***	***	<i>nd</i>	**	100 (9)
CZ-OHR	Mowing/Fertilization	***	***	*	*	***	*	*	ns	***	ns	80 (10)
SE-BAL	Grazing/Abandonment	*	**	*	ns	ns	**	*	ns	<i>nd</i>	**	67 (9)
SC-SUT	Agriculture/Abandon.	ns	<i>nd</i>	***	ns	ns	ns	ns	**	***	**	44 (9)
FR-ERC	Mowing/Grazing/Fertil.	**	<i>nd</i>	**	*	*	ns	ns	ns	*	*+	66 (9)
FR-LAU	Mowing/Grazing/Fertil.	***	**	a	ns	***	***	***	ns	**	***	80 (10)
NO-BER	Grazing	ns	ns	ns	ns	ns	ns	ns	ns	ns	<i>nd</i>	0 (9)
% sites signif.		60 (10)	88 (8)	82 (11)	37 (11)	64 (11)	55 (11)	64 (11)	36 (11)	78 (9)	88 (8)	
Across-sites	Most used / Least used	***	***	***	ns	***	**	***	***	***	***	90 (10)
		+	+	+		-	+	-	+	-	+	

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). The last column gives the percentage of ecosystem/soil properties showing significant differences among treatments within a site (the number between brackets shows the number of ecosystem/soil properties available at that site), and the last line gives the percentage of sites in which a particular properties varies significantly among treatments (the number between brackets shows 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; $K_{\text{nat-lab}}$ (NIRS), decay rate assessed by Near Infra-Red 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 -/+.

<i>Site</i>	<i>Disturbance/resource treatments</i>	<i>AGBmass</i>	<i>AGTot dead</i>	<i>ANPP</i>	<i>K_{nat-lab} (NIRS)</i>	<i>Soil C</i>	<i>Soil N</i>	<i>Soil P-Olsen</i>	<i>NNI</i>	<i>PNI</i>	<i>Species richness</i>	<i>% prop. signif.</i>
IS-KDE	Grazing/Abandonment	***	***	***	***	ns	ns	*	*	**	***	80 (10)
PT-MER	Agric./Grazing/Aband.	+	+	+	-	*	a	***	ns	**	**	90 (10)
GR-LAG	Grazing/Abandonment	***	***	***	ns	***	***	**	*	***	***	90 (10)
FR-HGM	Abandonment	*	-/+	*	a	***	***	ns	ns	ns	**	70 (10)
GE-MNP	Grazing/Aband./Water	+	+	-	-	+	+	***	nd	nd	-	100 (8)
CZ-OHR	Mowing/Fertilization	***	***	***	*	a	***	***	nd	nd	***	60 (10)
SE-BAL	Grazing/Abandonment	ns	***	ns	**	ns	ns	***	a	***	***	60 (10)
SC-SUT	Agriculture/Abandon.	**	+	**_	-	***	**	a	ns	nd	*	89 (9)
FR-ERC	Mowing/Grazing/Fertil.	-	+	ns	-	+	-	*	ns	*	*	40 (10)
FR-LAU	Mowing/Grazing/Fertil.	a	ns	*	ns	ns	ns	*	*	**	**	60 (10)
NO-BER	Grazing	ns	*	ns	***	ns	ns	*-	a-	ns-	+	50 (10)
		nd	+	nd	-	ns	ns	ns	nd	nd	-	20 (5)
			+								+	
% sites signif.		70 (10)	80 (10)	70 (10)	64 (11)	55 (11)	64 (11)	81 (11)	56 (9)	75 (8)	100 (11)	











