

Broad-scale variation of fungal-endophyte incidence in temperate grasses

María Semmartin*, Marina Omacini, Pedro E. Gundel and Ignacio M. Hernández-Agramonte

IFEVA – Facultad de Agronomía, Universidad de Buenos Aires, CONICET, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina

Summary

1. The strength of many interactions between plants and other organisms changes across regional gradients. For example, the relevance of plant-herbivore interactions increases with primary production. Likewise, biotic interactions collectively become more intense from the poles to the equator. Yet, the regional variation of the interaction between grasses and systemic fungal endophytes, which provide resistance to biotic and abiotic environmental factors (i.e. herbivory and drought), is poorly understood.

2. We compiled 1008 records of the incidence level of fungal endophytes (*Epichloë*, Ascomycetes: Clavicipitaceae) on wild populations of 48 cool season grasses, encompassing 10 biomes across a broad latitudinal expanse and primary production gradient. Symbiosis incidence was analysed as a function of mean primary production, precipitation, temperature and latitude of each site, which in turn were obtained from climatic and satellital sources.

3. Across a 30-fold variation of mean primary production, average symbiosis incidence increased from 20% to 70%. The pattern became stronger when the analysis was restricted to the single grass genus *Festuca*, which accounted for half of the total data.

4. The number of grass populations showing no symbiosis incidence (0%) decreased as primary production increased, whereas those with 100% of incidence increased.

5. Primary production at the regional scale was negatively correlated with latitude but positively with mean annual temperature and precipitation. Symbiosis incidence was similarly correlated with latitude and temperature, and it was not with mean annual precipitation.

6. *Synthesis*. Different descriptors of this grass-fungus symbiosis show that average incidence in wild populations world-wide increases with mean primary production. As at large spatial scales herbivory and temperature increase and aridity decreases with primary production, our results suggest that, at broad-scales, these biotic and abiotic factors may be important drivers of the symbiosis success.

Key-words: determinants of plant community diversity and structure, *Epichloë*, herbivory resistance, mutualisms, *Neotyphodium*, primary production, regional pattern

Introduction

Symbioses between plants and a variety of microorganisms remarkably impact on ecosystem properties such as nutrient cycling and primary production (Klironomos *et al.* 2000; Rudgers, Koslow & Clay 2004; Thrall *et al.* 2007). Probably because of their role in both agro ecosystems and natural nutrient-poor environments, two groups of root symbionts, mycorrhizal fungi and nitrogen fixing bacteria, have been the most studied plant symbionts (Van der Heijden & Sanders 2002; Douglas 2010). Conversely, the systemic Clavicipitaceous leaf

fungal endophytes of grasses have been significantly less considered (Omacini *et al.* 2012). These endophytes profoundly affect grass population dynamics and adaptation to changing ecological environments, with ultimate consequences on plant communities and ecosystems processes (Saikkonen *et al.* 1998; Omacini *et al.* 2001, 2012; Clay & Schardl 2002; Rudgers, Koslow & Clay 2004; Gibert & Hazard 2013).

A challenging goal of ecology is to identify patterns of variation of ecosystem properties across broad spatial scales and to understand their controls (Thrall *et al.* 2007; Schemske *et al.* 2009). Thus, ecologists have studied the strength of interactions between plants and other organisms across

*Correspondence author: E-mail: semmartin@agro.uba.ar

regional gradients. Biomass and consumption rate by mammal herbivores are positively correlated with primary production along a broad gradient of primary production across different biomes and continents (McNaughton *et al.* 1989; Oosterheld, Sala & McNaughton 1992). Likewise, the fraction of animal-dispersed plants increases from high to low latitudes (Moles *et al.* 2007). In contrast, the variation of plant interactions with microorganisms at the global scale is less clear (Thrall *et al.* 2007; Rodriguez *et al.* 2009). For example, the predicted lower prevalence of arbuscular mycorrhizal fungi as soil organic matter increases (Read 1991) is not confirmed by empirical data at the global scale (Treseder & Cross 2006).

Clavicipitaceous fungal endophytes establish long-lasting or persistent symbiotic associations with many cool season grasses (Pooideae subfamily) (Saikkonen *et al.* 1998; Clay & Schardl 2002; Rodriguez *et al.* 2009; Schardl 2010). They were recently unified under the genus *Epichloë* (Leuchtmann *et al.* 2014) including *Neotyphodium*. This group of endophytes is estimated to form symbiosis with up to 20–30% of the approximately 10 000 grass species (Poaceae), although < 4% have been described (Leuchtmann 1992). In contrast to the most common, horizontally transmitted fungal endophytes found in most plant families (Class 1 endophytes in Rodriguez *et al.* 2009) most *Epichloë* species reproduce vegetatively and transmit vertically: hyphae grow into the developing seeds of the host without causing symptoms of disease (Clay & Schardl 2002; Rodriguez *et al.* 2009; Schardl 2010). Therefore, the fungal endophyte depends on the plant for growth, reproduction and dispersal through seeds (Gundel *et al.* 2008; Rodriguez *et al.* 2009; Schardl 2010; but see Tadych *et al.* 2012; Oberhofer & Leuchtmann 2014).

The endophyte provides some benefits to the host (Ewald 1987; Saikkonen *et al.* 2004). Plants with endophytes show a higher ability to withstand several types of stress (Malinowski & Belesky 2000). But most importantly, the grass-endophyte symbiosis is an example of a defensive mutualism (Clay 1988). Indeed, in some species, bioactive fungal alkaloids protect the host against vertebrate and invertebrate herbivores (Clay 1988, 1990; Rodriguez *et al.* 2009; Schardl 2010).

Despite the great effort made during the last two decades to increase our understanding of this symbiosis, the factors driving its distribution and abundance are still under debate (Rudgers *et al.* 2009; Gundel, Rudgers & Ghersa 2011). The proportion of symbiotic individuals in a grass population (i.e. incidence) varies significantly from 0% to 100% (Clay 1990; Clay & Schardl 2002; Rudgers *et al.* 2009). This variation may be the result of variable cost/benefit or antagonism/mutualism continuum across environments (Saikkonen *et al.* 1998, 2004; Faeth 2002). In addition, low resource conditions may impair the endophyte transmission to the next plant cohorts and so destabilise the mutualism (Gundel *et al.* 2010; Gundel, Rudgers & Ghersa 2011). Therefore, endophyte incidence within a population may vary according to variations in fitness between symbiotic and non-symbiotic plants, in endophyte transmission and in migratory processes (Ravel, Michalakakis & Charnet 1997; Saikkonen, Ion & Gyllenberg 2002; Afkhami & Rudgers 2008; Gundel *et al.* 2008, 2010;

Gundel, Rudgers & Ghersa 2011). Further, knowledge about the broad-scale patterns of this symbiosis should provide a better understanding of the ecological factors controlling its success.

The studies on the broad-scale patterns of endophyte incidence at a regional scale are scarce, often contradictory, and focused on a reduced number of cultivated forage species. Endophyte incidence on a perennial grass (*Lolium* spp.) was higher in Mediterranean regions than in Northern Europe, suggesting an endophyte-mediated tolerance to drought (Lewis *et al.* 1997; Gibert *et al.* 2012). However, Afkhami (2012) reached the opposite conclusion after finding more grass genera and species known to form the symbiosis in non-Mediterranean than in Mediterranean environments. Finally, the variation of endophyte incidence along altitudinal and grazing gradients in Northern Europe varied among species (Bazely *et al.* 2007; Granath *et al.* 2007).

Here, we reviewed the empirical evidence on endophyte incidence in wild grass populations and investigated its relationship with mean primary production, latitude and other environmental variables. Primary production integrates the effect of other environmental features such as precipitation and temperature and correlates with latitude, altitude and continentality (Gaston 2000). It is also a measure of the energy and resource availability for both plants and heterotrophic organisms (McNaughton *et al.* 1989; Oosterheld, Sala & McNaughton 1992; Wootton & Power 1993; Bertness & Callaway 1994; Thrall *et al.* 2007; Schemske *et al.* 2009). Thus, we chose primary production to characterise the broad-scale patterns of the symbiosis. We compiled a data set from 27 published articles on 48 grass species known to form symbiosis with endophytes. The data set encompassed 10 biomes world-wide from deserts to tropical grasslands and a 30-fold variation of mean annual net primary production.

Materials and methods

We gathered data from surveys of endophyte incidence in wild grass populations around the world, published in journals compiled by Scopus (<http://www.scopus.com>). For each data point, we gathered data on net primary production, mean annual precipitation and temperature.

GRASS-ENDOPHYTE SYMBIOSIS DATA

We searched the Scopus data base for articles published until 2011 with different combinations of the terms *grass*, *frequency*, *incidence*, *Neotyphodium*, *Acremonium* and *Epichloë* in the title, abstract or keywords. After checking for the effectiveness of the search criteria, we reviewed the 169 resulting articles and selected those fulfilling two criteria: (i) the article provided detailed and quantitative information on endophyte incidence (in seeds, stems or both) and geographic location of the collection site; (ii) samples corresponded to field data of wild populations. We considered a population as wild when there was no indication that it had been sown in the collection site. Therefore, data from cultivated pastures, botanical gardens or experiments were discarded. We took special care to include the old articles in which the name *Acremonium* was used for *Neotyphodium*/*Epichloë*

endophytes (e.g. Lewis *et al.* 1997) and discard the ones on true *Acremonium*.

After this screening, only 27 articles remained in the data base (see Table S1 in Supporting Information). As we recorded the endophyte incidence for each species and collection site, the data base had as many entries as combinations of species and collection sites. The final data set had 1008 entries from 734 sites and 48 plant species (Table S1). A group of 167 entries corresponded to 2 widely cultivated forage species (*Lolium perenne* and *Festuca arundinacea*) that were naturalised in the collection site. Because several countries prohibit the commercialisation of endophyte-infected seeds of these species, and wild populations may receive seed influx from pastures we checked for bias by excluding *L. perenne* and *F. arundinacea* from the data set. As the results remained the same, we included both species.

We classified each species as exotic or native taking into consideration its origin in relation to the collection site (Tropicos data base from the Missouri Botanical Garden, <http://tropicos.org>). Most data were from native grass populations (905 entries). The small fraction of entries that corresponded to exotic species (103) was naturalised populations of seven European species (*Lolium multiflorum*, *L. perenne*, *L. rigidum*, *Festuca arundinacea*, *F. altaica*, *Poa annua* and *Phleum alpinum*) collected in grasslands of North and South America. This study does not include species that were never reported to form this symbiosis because they were irrelevant to our analysis (Leuchtmann 1992).

Geographical locations were transformed into exact latitude and longitude coordinates from either the reference to those coordinates in the original study, information obtained from the corresponding authors, or references to other geographical features, such as towns or specific neighbouring locations.

ENVIRONMENTAL DATA

For each collection site, we estimated net primary production ($\text{g C m}^{-2} \text{ y}^{-1}$), mean annual precipitation (mm) and mean annual temperature ($^{\circ}\text{C}$). Primary production was independently estimated from remote sensing (Zhao *et al.* 2005), whereas the rest were estimated from meteorological stations (Hijmans *et al.* 2005). Net primary production was obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) primary production products (Zhao *et al.* 2005) (MOD17A3) (pixel resolution = 1 km^2). For a 7-year period (2000–2006), we averaged all pixels included in a 1 km radius around each collection site. Pixels with null values (frequently denoting superficial water or urbanisation) were not averaged. Mean annual precipitation and temperature of each collection site were obtained from *worldclim*, a high spatial resolution data base (1 km^2) that integrates a 50-year period (1950–2000) from meteorological stations with at least 10 years of continuous data (<http://www.worldclim.org>). We also assigned to each collection site its vegetation type category (biome) (Olson *et al.* 2001).

The data set encompassed a 30-fold gradient of primary production and included 10 biomes in the following proportion (Fig. S1 and Table S1): temperate broadleaf and mixed forest (34% of data), tundra (19%), temperate grasslands, savannas and scrublands (17%), temperate and coniferous forest (11%), boreal forest, taiga (10%), Mediterranean forests, woodlands and scrublands (5%), deserts and xeric shrublands (2%), montane grasslands and shrublands (1%), tropical and subtropical savannas, grasslands, and shrublands (0.7%), tropical and subtropical coniferous forests (0.3%).

With these environmental variables, we do not pretend to characterise the environment at the time of sampling. Instead, we want to

assess the average differences among collection sites and their inter-annual variability. For instance, a descriptor of net primary production of different plant communities from a subhumid grassland showed that the spatial variability of primary production ranged between 5% and 35% during the growing season and its temporal variability among years ranged between 5% and 15% (Aragón & Oosterheld 2008).

DATA ANALYSIS

To study the patterns of endophyte incidence, we used generalised linear models (R Development Core Team 2010). The dependent variable was endophyte incidence. The data were partitioned in three ways: (i) the entries with extreme incidence (0% and 100%), (ii) the complete data set and (iii) the entries with incidence other than 0% and 100%. Additionally, we performed separate analyses for native and exotic species and for the most frequent genus (*Festuca*). We included here *F. arundinacea* because its denomination as subgenus *Schedonorus* (*S. arundinaceus*) is still under debate (Catalán *et al.* 2007).

The selection process of explanatory variables (primary production, latitude, mean annual temperature and precipitation) started with a correlation analysis between pairs of variables (Crawley 2007; Zuur *et al.* 2009). Since we focused on primary production as an integrative variable of others (e.g. precipitation, temperature) and as a surrogate of local environmental quality, we first eliminated all variables highly correlated with primary production. Thus, mean annual precipitation and temperature were withdrawn (Pearson $r = 0.91$, and $r = 0.62$, respectively), and latitude was retained ($r = -0.29$). The model selection process started comparing the more complex model (with the interaction between primary production and latitude) against more simple models through analyses of deviance (Crawley 2007). We also regressed primary production against latitude, mean annual temperature and mean annual precipitation, and endophyte incidence of the complete data set with temperature and precipitation.

Results

The two extreme values of incidence (0% and 100%) together represented nearly 40% of the data (Fig. 1). Intermediate classes accounted for the remaining data and those between 1%

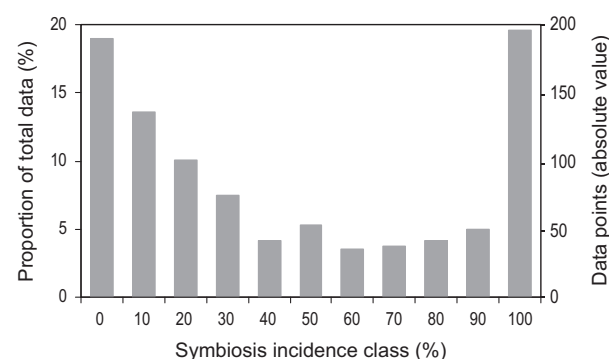
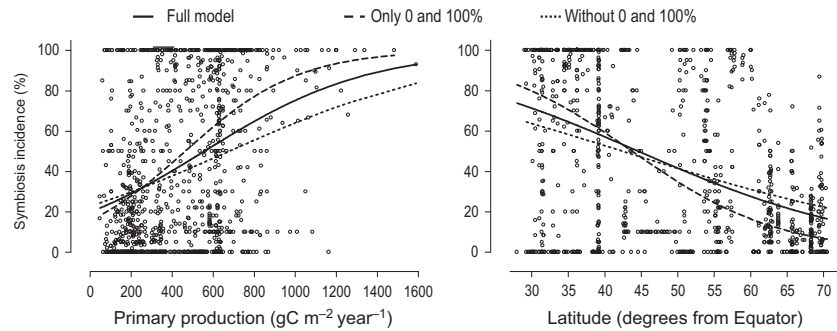


Fig. 1. Frequency distribution of different incidence levels of *Epichloë* fungal endophytes in grass populations. Both extreme classes only included populations with 0% and 100% of symbiosis incidences, respectively, whereas the other classes include 10% ranges of incidence (e.g. 10 = 1 to 10, 20 = 11 to 20).

Fig. 2. Relationship between *Epichloë* endophyte incidence, primary production and latitude. The regression lines show the predictive values according three different data subgroups: *Complete*, the entire data set; *Only 0% and 100%*, only the extreme incidence values; *Without 0% and 100%*, the entire data set excluding the extreme incidence values.



and 30% of incidence resulted slightly more frequent than the others (Fig. 1).

For three partitions of the data set, endophyte incidence varied with primary production and latitude. Endophyte incidence was positively correlated with primary production ($F_{1,1006} = 83.4$, $P < 0.001$) and negatively with latitude ($F_{1,1005} = 89.4$, $P < 0.001$) (Fig. 2, 'Full model'). Furthermore, the proportion of grass populations with 100% incidence increased with primary production and decreased with latitude, whereas the proportion of populations with 0% of incidence showed the opposite pattern (Fig. 2, 'Only 0% and 100%' model). Finally, the analysis excluding the extreme values of incidence ('Without 0% and 100%' model), discarding any potential bias due to the greater contribution of extreme values to the total data set revealed in Fig. 1, also showed a pattern consistent with the others.

Separate analyses explored the effect of the origin and phylogeny on the patterns just described. Individual analysis for native ($n = 905$) and exotic ($n = 103$) grass species showed similar patterns to the pooled (native + exotic) analysis with the exception that for the exotics latitude was not significant (Table 1). When the data set was restricted by a phylogenetic criterion to the most reported genus (*Festuca*), endophyte incidence exhibited a stronger positive and negative relationship with primary production ($F_{1,473} = 104.9$, $P < 0.0001$) and latitude ($F_{1,472} = 78.4$, $P < 0.0001$), respectively (Fig. 3). There were 15 *Festuca* species out of the 48 species included in this study. They were widely distributed along the gradients here considered and accounted for approximately half of the data set ($n = 475$). In addition, the group of species other than *Festuca* had a similar pattern for both primary production and latitude ($n = 533$, $P < 0.0001$ data not shown).

Table 1. Parameters describing the relationship between *Epichloë* endophyte incidence (%) of native and exotic species with primary production and latitude

Explanatory variable	Natives ($n = 905$)		Exotics ($n = 103$)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Primary production ($\text{gC m}^{-2} \text{ year}^{-1}$)	55.97	< 0.0001	13.7	< 0.0001
Latitude (degrees from equator)	4.22	< 0.001	0.99	0.14

The regional variation of net primary production was associated with the variation of several environmental factors including latitude (Table 2). Nevertheless, symbiosis incidence was not necessarily correlated with them (Table 3). Primary production was positively related to both mean annual temperature and precipitation and negatively to latitude (Table 2), whereas symbiosis incidence was positively related to mean annual temperature but not to mean annual precipitation (Table 3).

Discussion

The incidence of fungal endophytes in wild grass populations increased along a 30-fold gradient of primary production and decreased with distance from the equator. This pattern was even stronger when the data set was limited to a more homogeneous phylogenetic plant group, species of the *Festuca* genus. Endophyte incidence also increased with mean annual temperature, whereas it was not significantly related to mean annual precipitation. Because primary production was positively correlated with mean annual temperature and precipitation, our results suggest that, at the global scale, this

Fig. 3. Relationship between *Epichloë* endophyte incidence, primary production and latitude of the most frequently documented ($n = 475$) plant genus, *Festuca*.

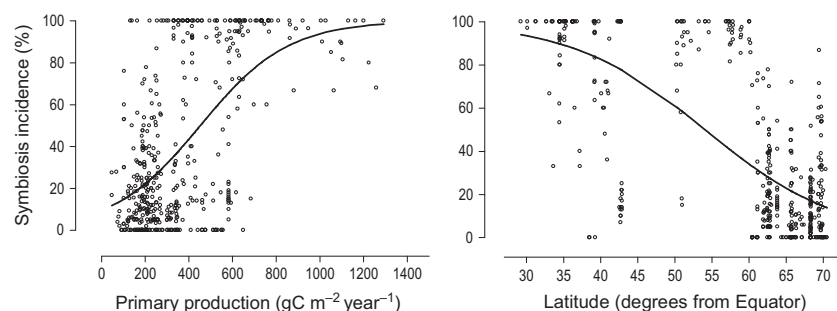


Table 2. Parameters describing the relationship of primary production ($\text{gC m}^{-2} \text{ year}^{-1}$) with other environmental variables. Confidence intervals for constant and slope are indicated in parentheses ($n = 734$)

Regressor variable	Constant	Slope	R^2	P
Latitude (degrees from equator)	850.9 (785.8; 916.1)	-8.1 (-9.37; -6.86)	0.18	0.0001
Temperature ($^{\circ}\text{C}$)	382.2 (367.3; 397.1)	25.6 (23.5; 27.7)	0.44	0.0001
Mean annual precipitation (mm)	149.8 (110.2; 189.5)	0.43 (0.38; 0.49)	0.26	0.0001

Table 3. Parameters describing the relationship between *Epichloë* endophyte incidence and other environmental variables. Confidence intervals for constant and slope are indicated in parentheses

Regressor variable	Constant	Slope	R^2	P
Temperature ($^{\circ}\text{C}$)	37.6 (28.1; 47.3)	2.4 (1.1; 3.8)	0.65	0.003
Mean annual precipitation (mm)	31.5 (7.8; 55.2)	0.01 (-0.01; 0.05)	0.08	0.22

symbiosis is benefited by high resource availability and ecosystem carrying capacity.

Revealing these broad-scale patterns is a major step in our understanding of this symbiosis. Until now, the only certain pattern was that the grass-endophyte symbiosis was restricted to temperate and cold habitats. There was also a controversy on the patterns in Mediterranean stressful ecosystems (Lewis *et al.* 1997; Afkhami 2012; Gibert *et al.* 2012). Our results revealed that average incidence increases from 20% to 70% along a wide gradient of primary production ($50\text{--}1600 \text{ g C m}^{-2} \text{ y}^{-1}$) that included 10 biomes and a broad latitudinal expanse (from $\sim 70^{\circ}$ to 28° in the Northern Hemisphere and from 55° to 25° in the Southern Hemisphere) (Fig. S1).

The negative relationship between grass-endophyte incidence and latitude coincides with regional patterns of interactions between plants and other organisms. The abundance per unit of leaf area of a variety of foliar fungal endophytes decreased in trees from the tropical forests of Panama to the Canadian arctic (Arnold & Lutzoni 2007). Other biotic interactions, such as herbivory, predation, cleaning mutualisms, etc., also decrease with latitude (Schemske *et al.* 2009). Interestingly, our pattern emerged from the narrower latitude range where this symbiosis takes place. The vertically transmitted grass endophytes studied here are not found in tropical ecosystems, even in the potential hosts (C_3 grasses) inhabiting the tropical forest understorey (Higgins *et al.* 2011). Therefore, our pattern is consistent with those already described for other biotic interactions encompassing wider latitudinal ranges.

Latitude, as a proxy for a wide range of bioclimatic factors, does not stand as a meaningful variable (Gaston 2000). Our results showed that latitude was significantly correlated with primary production, which in turn was strongly correlated with mean temperature and precipitation (Table 2). In other words, changes in relevant environmental variables related to latitudinal variation were largely captured by primary production. Likewise, tree individuals from riparian forests of Central Arizona located along a fixed latitude had greater

endophyte incidence as mean annual precipitation, and probably primary production, increased (Lau, Arnold & Johnson 2013). The strength of the interaction between plants and vertebrate herbivores also increases across regional gradients of primary production. World-wide, grazing intensity and wild and domestic herbivore biomass increase exponentially with primary production (McNaughton *et al.* 1989; Oesterheld, Sala & McNaughton 1992; Oesterheld, Di Bella & Herdiles 1998). All this evidence suggests that the patterns of biotic interactions between plants and other organisms follow carrying capacity gradients.

The broad-scale pattern provided here helps to disentangle the relative role of abiotic stresses as selective forces for this symbiosis (Thrall *et al.* 2007; Rodriguez *et al.* 2009). The lower endophyte incidence in drier, colder and less productive sites suggests that harsh environments impair rather than promote the symbiosis. This pattern was not expected from the experimental evidence on higher drought tolerance of endophytic plants (Malinowski & Belesky 2000) and regional European patterns (Lewis *et al.* 1997). Less productive systems also experience greater year-to-year variability of precipitation and plant production (Le Houerou, Bingham & Skerbek 1988; Oesterheld, Di Bella & Herdiles 1998). As a consequence, they have a higher chance of events with extremely low temperature and water availability. Coincidentally, extremely low temperature reduces endophyte transmission (Ju *et al.* 2006). Therefore, harsh conditions may shift the mutualism into parasitism, reduce the relative fitness of grass hosts and/or reduce endophyte transmission (Saikkonen *et al.* 1998; Afkhami & Rudgers 2008; Gundel *et al.* 2008; Gundel, Rudgers & Ghera 2011). In conclusion, the scale of analysis affects our interpretation of the symbiosis impact on the host from an evolutionary perspective (Saikkonen *et al.* 2006).

The biotic interactions between grasses and herbivores might also account for the pattern documented here. Because at broad regional scale herbivory increases with primary production (McNaughton *et al.* 1989; Oesterheld, Sala & McNaughton 1992), the higher endophyte incidence in more productive systems is consistent with the resistance to

herbivory conferred by the endophyte to host plants. The role of the endophyte as a defensive mutualist supported by the regional scale pattern agrees with experimental evidence at smaller scales (Omacini *et al.* 2001; Saikonen, Saari & Helander 2010).

In conclusion, this study showed for the first time a broad-scale pattern for the distribution and abundance of the grass-endophyte symbiosis. It agrees with early predictions of under what conditions defence in endophytes should be more probable (Faeth 2002). Different descriptors of this grass-fungus symbiosis showed that incidence in wild populations worldwide increases with mean primary production. Because at large spatial scales herbivory and temperature increase and aridity decreases with primary production, our results suggest that, globally, these biotic and abiotic factors may be important drivers of the symbiosis success. The broad-scale pattern here described should provide a framework for novel hypotheses and further studies on the ecological factors controlling this grass-fungal symbiosis at finer spatial scales, such as grazing regime (Koh & Hik 2007), altitude (Bazely *et al.* 2007; Granath *et al.* 2007) or fertility (Malinowski & Belesky 2000). Field manipulative experiments with wild grass populations, coordinated in a networking survey including herbivore exclusion and water shortage across productivity gradients, are crucial to reveal the relative importance of each driver of this symbiosis success.

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Data accessibility

Data deposited in the Dryad repository (Semmartin *et al.* 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Map with the global distribution of the *Epichloë* endophytes.

Table S1. Distribution of the studied plant species and bibliographic references.