Chapter 3
Evidences of Bryophyte Allelochemical Interactions: The Case of *Sphagnum*

Geneviève Chiapusio, Vincent E. J. Jassey, M. Iftikhar Hussain and Philippe Binet

Abstract In most terrestrial ecosystems, allelochemical interactions are involved in vascular plants. Nevertheless, bryophytes represent also a crucial group found in many ecosystems with a specific morphology and physiology (e.g. secondary metabolism). Among bryophytes, *Sphagnum* genus is of particular interest because they form a dense homogeneous carpet which is slowly decomposed (the peat) in peatlands. Such ecosystems represent a terrestrial sink of carbon and so are crucial to be studied, especially under a climate change. Objectives of this chapter were (1) to synthesize current bryophyte allelochemical interactions and (2) to illustrate recent research on *Sphagnum* with the case of *Sphagnum fallax* phenolics (production and degradation) recovered in a french peatland. The top layer of living *Sphagnum* represents the active allelopathic part where water-soluble phenolics were mostly recovered. Their concentrations were found to change along the seasons. The transformation of phenolic compounds is performed by an enzymatic system O_2 (phenoloxidases) or H_2O_2 (peroxidases) dependent. *Sphagnum*-peroxidases constituted the main oxidative system and fungal phenoloxidases were proposed to be regulated by phenolics. Moreover, *Sphagnum* was able to regulate its secondary metabolism under a climate forcing by decreasing its phenolic concentrations. Allelopathic potential of *Sphagnum* phenolics was stated with their role in the microdistribution of associated *Sphagnum* microorganisms. Finally, *Sphagnum* extracts also strongly delayed *Pine* and *Lolium* germination seeds and inhibited *Lolium* radicle growth and delayed *Raphanus* and *Pinus* radicles. Ecological and agronomic perspectives of *Sphagnum* extracts are discussed.

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3.1 Introduction

Since the early 1970s, research efforts on allelopathy have increased considerably. Because allelopathy meets current societal demands for environmentally sound approaches to agriculture, there is a growing interest in discovering, and understanding allelochemicals produced by plants. One way to identify allelopathic interactions is to study plants in their ecosystems. Numerous ecosystems have been studied through allelochemical interactions such as forests (Souto et al. 2000) or grasslands (Viard-Crétat et al. 2009). They involved mostly gymnosperms and angiosperms rather than nonvascular plants. Bryophytes represent one successful group of plants in terms of geographical distribution on all continents, habitat diversification, and number of species (Slack 2011). Even if bryophytes are known to produce chemicals which make mosses very unattractive for herbivores, little is known about the different roles of phenolics excreted by mosses in their surroundings. Allelopathy represents then one potential ecological interaction among all complex interactions involved in the functioning of ecosystems.

Among terrestrial ecosystems, peatlands are dominated by bryophytes of *Sphagnum* genus which forms a homogeneous carpet. The accumulated peat is mainly dominated by remnants of *Sphagnum*. Indeed, peatlands are characterized by the ability to store atmospheric carbon in the long-term accumulation of partially decomposed organic matter in form of decaying vegetation. The accumulation of organic matter in peatland is the result of production and decay of *Sphagnum* tissues with recalcitrant carbon such as polyphenols. The transformation of phenolic compounds is performed by an enzymatic system O_2 (phenoloxidases) or H_2O_2 (peroxydases) dependent. The interplay polyphenols/phenoloxidases is then a key process in peatland functioning. Recent research on the organo-chemical composition of *Sphagnum* clarified the ability of *Sphagnum* to outcompete other plants for light by creating acidic nutrient poor cold and anoxic conditions (Van Breemen 1995). The role of polyphenols on peatland functioning is mostly associated with the low decomposition of peat, which is of great importance for the peat storage function (Verhoeven and Liefveld 1997). Surprisingly, the exact role of phenolics remains understudy in growing *Sphagnum* plants. Moreover, a few studies focused on allelopathic interactions (Michel et al. 2011).

Recently, boreal peatland is of particularly interest, because it currently represents a terrestrial sink of carbon with approximately one-third of the world's organic carbon (390–455 Pg) (Gorham 1991; Moore 2002). The expected increase of air temperatures in boreal regions is predicted to lead to a destabilization of peatland carbon stores (Smith et al. 2004; Strack 2008). *Sphagnum* represents the crucial plants to study the effect of elevated temperature on peatlands. Most of the studies aim at understanding effects of temperature on the peat decomposition, but not on living *Sphagnum*. The study of *Sphagnum* phenolic metabolism under a climate forcing remains a challenge even if difficult and complex.

In this chapter, we present the main characteristics of bryophytes involved in allelochemical interactions. Then, the case of *Sphagnum* genus will fully illustrate why bryophytes is of interest to allelopathy based on their phenolic production, degradation, and variation.

3.2 Terrestrial Bryophytes

3.2.1 Morphology

Bryophytes represent an independent branch of the plant kingdom with more than 16,000 species, taking up a position between the algae and the pteridophytes (Slack 2011). From a taxonomic point of view they are divided into three classes namely hornworts (Anthoceratae), liverworts (Hepaticae), and mosses (Bryatae). Bryophytes lack the roots, the xylem, and the phloem of vascular plants. The dominant stage is the haploid green autotrophic gametophyte rather than the diploid sporophyte which remains parasite of the gametophyte. Bryophytes play a fundamental ecological role in many terrestrial ecosystems especially in rain forest and cold biomes where they are abundant (Tan and Pocs 2000). Many species are able to live in nutrient poor conditions and are adapted to respond physiologically to intermittent periods favorable to photosynthesis (Slack 2011).

3.2.2 Allelochemical Interactions

Studies in various plant communities as well as greenhouse experiments have demonstrated that germination and/or seedling emergence of vascular plants are affected by mosses (Asakawa 1990). The effect may be either positive or negative depending on the habitat and the species. The positive effects are generally explained by the moisture conditions favorable to germination and the negative ones by the low light intensity, the drier microhabitat and allelopathy (Zamfir 2000). Thus, interactions between bryophytes and vascular plants comprise a large spectrum of relations including resource competition, suppression, facilitation, and allelopathy. Among these effects allelopathy seems to be the most controversial mostly because sparse experiments were conducted and among them, differences were noticed between greenhouse and field experiments (Soudzilovskaia et al. 2011). Nevertheless, original allelochemical interactions were revealed either with native or laboratory test species (Table 3.1). In boreal ecosystems, Soudzilovskaia et al. (2011) assumed that bryophytes exclusively affect germination and very early establishment but do not influence the fitness of established seedlings. Nevertheless, nothing is much known about the importance of interspecific differences among bryophytes with respect to their effect on vascular plants or the functional traits responsible for such effects.

Table 3.1 Examples of laboratory experiments on allelochemical interactions between Bryophytes (donor plants) and vascular plants (target plants)

Bryophytes "donor plants"	Vascular plants "target plants"	Allelochemical effects	References
Hylocomium splendens, Pleurozium schreberi, Dicranum scoparium, Polytrichum strictum, Barbilophozia lycopodioides, Ptilidium ciliare	*Betula pubescens, Pinus sylvestris, Epilobium angustifolium, Solidago virgaurea, Festuca ovina*	Germination and growth inhibition	Soudzilovskaia et al. (2011)
Rhynchostegium pallidifolium	Cress, lettuce, alfafa	Growth Inhibition	Kato-Noguchi et al. (2010)
Sphagnum palustre, Racomitrium japonicum, Dicranum japonicum, Hypnum plumaeforme	Lettuce	Growth Inhibition	Tsubota et al. (2006)
Hypnum plumaeforme		Momilactone A and B (growth inhibitors)	Nosaki et al. (2007)
Dicranum scoparium, Racomitrium canescens, Homalothecium lutescens, Hylocomium splendens, Hypnum cupressiforme, Rhytidiadelphus triquetrus	*Arenaria serpyllifolia, Veronica spicata, Filipendula vulgaris, Festuca ovina*	Seedling emergence inhibition/or not specific to each species	Zamfir (2000)
Campylopus introflexus	*Calluna vulgaris*	No effect on germination and growth	Equiha and Husher (1993)

3.2.3 Bryophyte Secondary Metabolites

Recent analytical techniques allow characterizing numerous natural products from bryophytes. Phenolics or polyphenolic compounds are known to represent a main class of secondary metabolites synthesized by plants (Grooss et al. 1999). They are involved in physiological plant processes (e.g. germination) but also in plant chemical interactions (Hattenschwiler and Vitousek 2000). Compared to other plants, the chemical investigation of bryophytes is a rather young discipline. There are several reasons for this: it is difficult to collect larger amounts of material, fields cultivation is not practicable and laborious procedure to have pure species (Becker 2000). Despite these difficulties, a large number of natural products, some of them with novel and unique skeletons, have been isolated from bryophytes during the past 30 years.

Because bryophytes are nonvascular plant, their secondary metabolism is limited/different compared to vascular plants. Even if flavonoids remain one of the most studied group, only a small percentage has been studied in details because most of the effort having been devoted to species of Marchantiales, Jungermanniales and Bryales (Markham 1990). Remarkable diversity of terpenoids has also been showed to be produce by liverworts (Geiger 1990). Nevertheless, investigations directed to the biosynthesis of such compounds in nonvascular plants are still rare compared to the numerous studies on terpenoid metabolism in vascular plants. Another example is the still controversial presence of lignin in bryophytes, because degradation's studies on bryophyte tissues did not recovered the usual lignin degradation products (Geiger 1990; Ligrone et al. 2008). In view of the limited sampling, any generalization on bryophyte secondary metabolite is then difficult to make.

3.3 The Case of *Sphagnum*

Among Bryophyte classes, *Sphagnum* genus takes a particular place because of its fundamental ecological role in peatlands especially for the global sequestering carbon. Even if *Sphagnum* is the dominant plant in peats in terms of biomass and productivity, sparse data are focused on *Sphagnum* chemical interaction.

3.3.1 Sphagnum Secondary Metabolites

The *Sphagnum* genus, consisting of around 300 species, is worldwide distributed and is a dominant component of peat bog vegetation (Opelt et al. 2007). *Sphagnum* is morphologically characterized by two distinct layers : (1) the living layer or top layer called "the capitulum" where the *Sphagnum* grow by around 1 mm/year and where the main physiological process occurred and (2) the decaying layer or bottom layer where the decay constitutes the accumulation of peat (Fig. 3.1). The limit between the two layers is generally considered as 0–3 or 0–6 cm for the
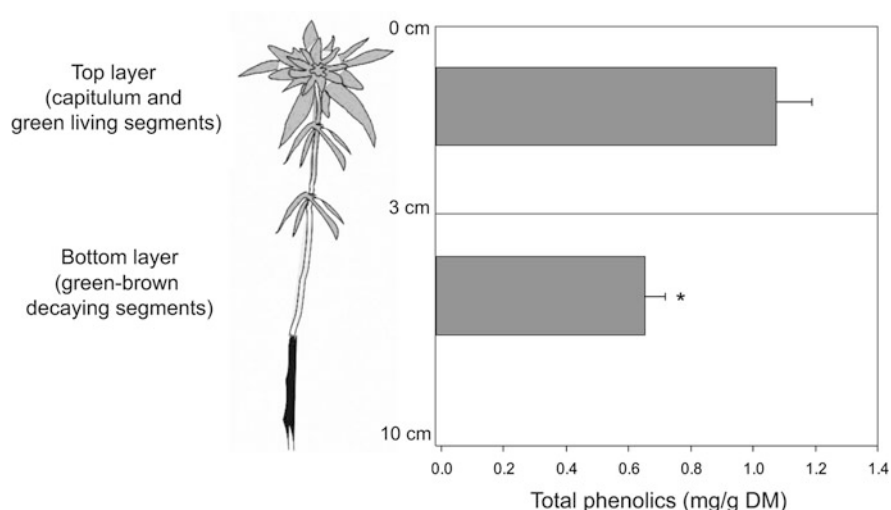
Fig. 3.1 Representation of *S. fallax* top and bottom layers with their respective total water-soluble phenolic concentrations expressed in mg g^{-1} DM. Mean ± S.E. ($n = 12$). *Asterisk* indicates significant differences between the two *Sphagnum* layers (Anova test $P < 0.05$) (adapted from Jassey et al. 2011a)

capitulum (0 from the top of the capitulum) and 3–10 or 6–10 cm for the bottom layer. The deepest layer (>10 cm) constitutes the peat soil itself.

All vascular plant organs contain varying amounts of chemicals with potential allelopathic effects. Nevertheless, here, only leaves represent the key living organ of *Sphagnum* because of their important biomass compared to stems. *Sphagnum* leaves are not fully protected from the surrounding environment because no cuticle is present. Moreover, *Sphagnum* leaves stored around 90 % of water in their hyaline cells. Thought that the exchange of water, from atmosphere, leachates and litter, a direct relation with *Sphagnum* surrounding environment occurs, and so ables easily chemical interactions.

The secondary metabolism of *Sphagnum* differs from vascular plants. For example, *Sphagnum* synthesizes specific polyphenolic compounds such as sphagnum acid (a cinnamic acid) synthesized via the Shikimate pathway (Rasmussen et al. 1995; Rudolph and Samland 1985). Another specific molecule is the flavonoid sphagnorubin, which is a red pigment which represents a natural constituent of cell wall of *S. magellanicum* (Tsutschek 1982).

3.3.2 Sphagnum Water Phenolic Production and Degradation

The first major group of secondary metabolite found in *Sphagnum* is the phenolics (Verhoeven and Liefveld 1997). To determine phenolic compounds produced by living sphagnum, the extraction method is crucial and depend on the goal of the
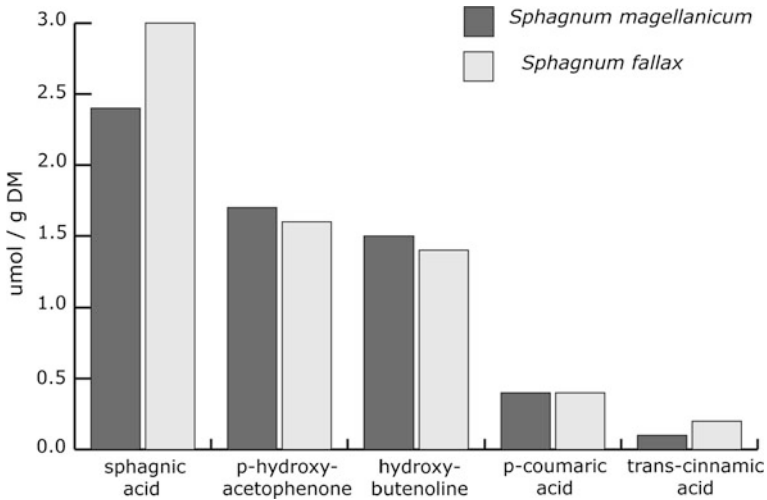
Fig. 3.2 Phenolic compounds quantified in *S. magellanicum* and *S. fallax* (adapted from Rasmussen et al. 1995)

study. Water to more polar solvents are commonly used and give different phenolic quantification recovery from the water extractable to the wall bound fractions. For example, *Sphagnum* species (*S. magellanicum, S. fallax, S. cuspidatum*) produced sphagnum acid, *p*-hydroxyacetophenone, hydroxy butenolide, *p*-hydroxybenzoic acid, *p*-coumaric acid, and trans cinnamic acid from 0.1 μmol g^{-1} DM up to 2.9 μmol g^{-1} DM (Fig. 3.2, Rasmussen et al. 1995). The phenolic profile was found to be species dependent (Opelt et al. 2007). To test allelopathic effect, the plant water extraction is commonly used.

In our work, *S. fallax* phenolics were extracted in cold water and quantified by using the Folin–ciocalteu reagent and gallic acid as standard (Jassey et al. 2011b). Phenolics were mainly recovered in the capitulum (1 mg g^{-1} DM) compared to the bottom layer (0.6 mg g^{-1} DM) (Fig. 3.1). These results clearly identified the active allelopathic part of *Sphagnum* as the top layer and the passive allelopathic part as the bottom layer. To conclude, the 0–10 cm *Sphagnum* layer gives a realistic allelopathic potential of *Sphagnum*.

The degradation of the recalcitrant polyphenolic plant residue represents a key process in the global carbon cycle of a peatland. A large diversity of extracellular enzymes, described as phenoloxidases, is involved in the degradation of polyphenolic compounds (Sinsabaugh 2010; Theuerl and Buscot 2010). Extracellular phenoloxidases are divided into phenoloxidases O_2 dependent (e.g. included laccases and tyrosinases; hereafter named phenoloxidases) and phenoloxidases H_2O_2 dependent (e.g. lignin and manganese peroxidases; hereafter named peroxidases). Generally, peroxidases are considered to be produced by basidiomycetes or ascomycetes but a recent work demonstrated that main peroxidase activities in an oak forest litter had a plant origin (Alarcón-Gutiérrez et al. 2009). According to

Fig. 3.3 Enzyme activities of two *S. fallax* layers 0–3 cm (*top*) and 3–10 cm (*bottom*) (October 2010): Phenoloxidases (**a**) and Peroxidases (**b**). Enzymatic activities were expressed in enzyme units (U) defined as one µmol of substrate oxidized per min^{-1} (peroxidase activities) or per h^{-1} (laccase activities) per gram of Dry Mass. Mean ± S.E. ($n = 6$). *Asterisks* indicate significant differences of enzyme activities among *Sphagnum* segments (Anova tests, $P < 0.05$) (adapted from Jassey et al. 2012)
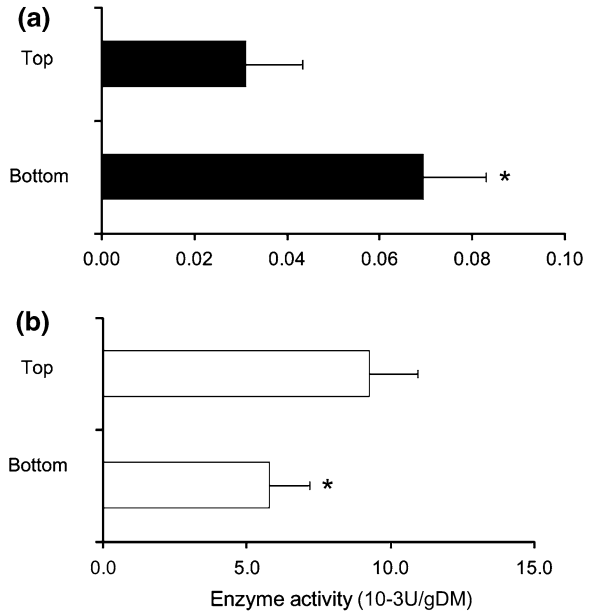

Sinsabaugh (2010), peatlands represent a particular focus for studies of phenoloxidases and peroxidases activities because of their large role in oxidizing recalcitrant carbon such as polyphenolics.

In a recent study, we measured variations of phenoloxidase and peroxidase activities in the *Sphagnum* peatland of Frasnes (Jura mountains, France). Extracellular enzymes were extracted using a specific extraction reagent (0.1 M $CaCl_2$ with 0.05 % Tween 80 and 20 g PVPP). After centrifugation, the extracts were concentrated in a cellulose dialysis with a 10 kDa molecular mass cut-off covered with polyethylene glycol (Jassey et al. 2011c). Enzymes activities were quantified using diaminofluorene, which represents a relevant oxidative substrate to quantify both plant peroxidases and fungal phenoloxidases in an enzymatic extract (Criquet et al. 2001; Jassey et al. 2012).

A negative correlation between total phenolics and fungal phenoloxidase activities was found (respectively, $r = -0.42$ and $r = -0.38$, $P < 0.01$) (Jassey et al. 2011c). Phenolics at high concentration could inhibit the oxidation activity of fungal phenoloxidases. A regulation of the fungal phenoloxidases by phenolics is then suggested. Ultimately, our results also reinforce the point that phenoloxidase/polyphenol interplay is especially critical to understanding peatland functioning.

Sphagnum peroxidases constituted the main oxidative system in *Sphagnum* peatlands, with values 1000-fold higher than fungal phenoloxidase activities. The highest level of peroxidase activities was measured in the top layer (0–3 cm), whereas fungal phenoloxidase activities were mainly detected in the deep layer (3–10 cm) (Fig. 3.3). Similar differences have been already recorded, but in the surface litter of forest with plant peroxidases 120-fold higher than fungal phenoloxidases (Alarcón-Gutiérrez et al. 2009).
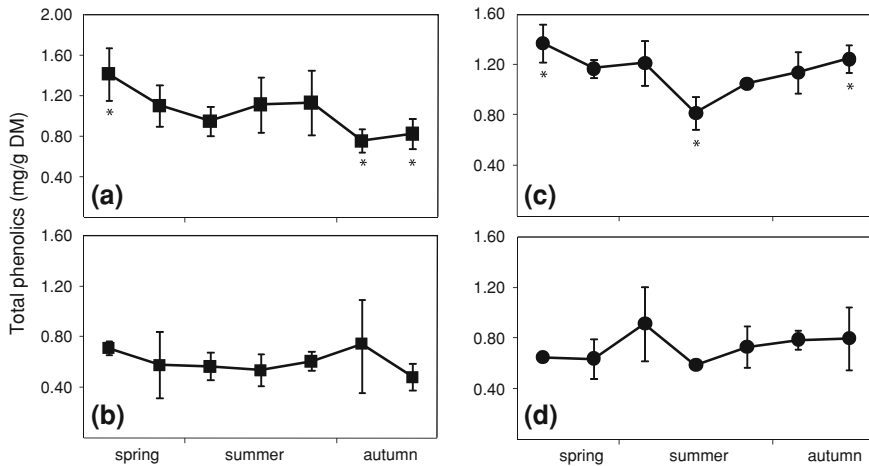
Fig. 3.4 Seasonal variations of water-soluble phenolic concentrations (expressed in mg g^{-1} DM) in two *S. fallax* layers (*top* **a**, **c** and bottom **b**, **d**) recovered in the bog (**a**, **b**) and fen (**c**, **d**) areas of the Frasne peatland. Mean $\pm$ S.E. ($n = 3$). *Asterisk* indicates significant difference among months (ANOVA tests, $P < 0.05$) (adapted from Jassey et al. 2011c)

3.3.3 Seasonal Variations of Phenolics in Ambient and Warming Environment

In most ecosystems, seasonal variations of phenolics have been quantified to characterized allelochemical potential (Gallet and Lebreton 1995). Surprisingly, in peatlands, such data are scarce (Bonnett et al. 2006).

Seasonal variations of phenolics, fungal phenoloxidases involved in their degradation were quantified in the Frasne peatland (Jura mountains, France) in two ecological conditions. Briefly, the first ecological area was a transition *Sphagnum*-dominated poor fen characterized by a flat homogeneous carpet of *S. fallax* (hereafter called "Fen") and the second one was a *Sphagnum* bog characterized by lawns of *S. fallax* and hollows of *S. magellanicum* associated with vascular plants (such as *Eriophorum vaginatum*, *Vaccinium oxycoccos*) (hereafter called "Bog") (Jassey et al. 2011c). Recovered *Sphagnum* phenolic concentrations were found to depend on the season with a decrease in summer for the bog area and in autumn for the fen area (Fig. 3.4). *Sphagnum* was able to regulate its secondary metabolism by decreasing its phenolic concentration when environmental conditions changed. Indeed, in summer in the bog area the air temperature increased and the *Sphagnum* moisture decreased inducing a decrease of total phenolics (Jassey et al. 2011c).

Because the increased decomposition rates in peatlands with global warming might increase the release of atmospheric greenhouse gases (i.e. CO_2), *Sphagnum*s are ideal plants for climate change indication. Most of the literature focused on the effects of temperature on the decomposition of litter from boreal peatlands. Cautious conclusions suggest that not all peatlands may provide a positive

Fig. 3.5 Seasonal variations of water-soluble phenolic concentrations (expressed in mg g^{-1} DM) in two *S. fallax* layers (*top* **a** and *bottom* **b**) recovered in controls and OTC in the fen areas of the Frasne peatland. Mean ± S.E. (*n* = 3). *Asterisk* indicates significant difference between control and OTC (ANOVA tests, $P < 0.05$) (adapted from Jassey et al. 2011c)
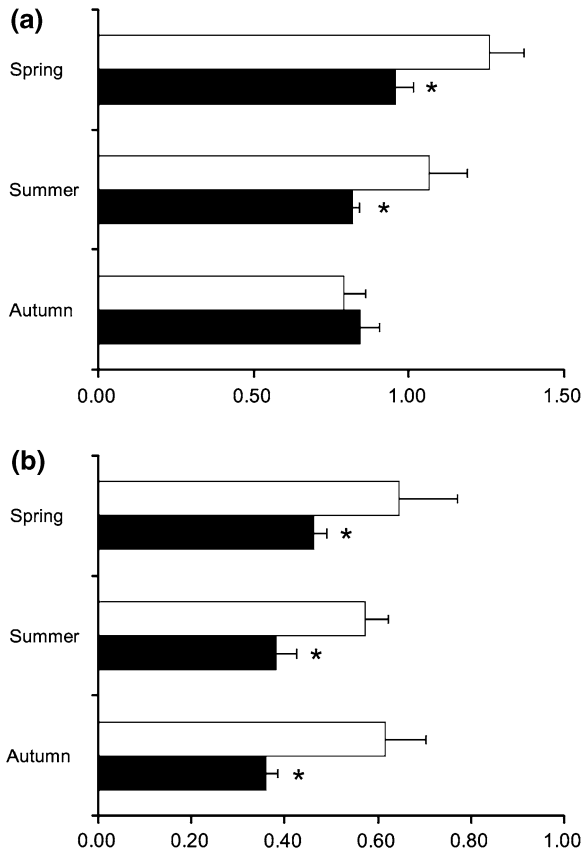
feedback to global warming (i.e. differences between the fen and bog areas) (Weltzin et al. 2003). Anyway, sparse data are available on living *Sphagnum* and their response to a global warming.

In the Frasne peatland, an increase of air and soil temperatures was passively achieved by placing hexagonal ITEX open-top-chambers over the vegetation. In spring and summer (may to September), the OTC's significantly increased the daily maximum air temperature by an average of 3 °C and the average air temperature by 1 °C (Jassey et al. 2011c).

In response to the climate forcing, the phenolic concentrations decreased in the fen area irrespective of the seasons (Fig. 3.5) but not in the bog area. *Sphagnum* carbon allocation between primary and secondary metabolism changed. *Sphagnum* diminished its allocation to polyphenols to favor growth or crucial synthesis to better survive (Veteli et al. 2007). Indeed, biomass allocation is an important plant trait that responds plastically to environmental perturbations. Our results also highlighted different responses of *Sphagnum* phenolics along the fen-bog gradient (Jassey et al. 2011c). More studies with long-term conclusion with more than 1 year are needed to draw complete conclusion on the effect on global warming on *Sphagnum*.

3.4 *Sphagnum* Allelopathic Interactions

Sphagnum-dominated peatlands were at one time erroneously believed to be devoid of microbial life because of the acid pH. In reality, a high diversity of microbial communities lives in *Sphagnum* mosses, among which decomposers (bacteria and fungi), primary producers (microalgae, diatoms and cyanobacteria), protozoa, and micrometazoa (ciliates, testate amoebae, rotifers, and nematodes). These microorganisms are of greatest interest in peatland functioning because they act on nutrient cycling, mainly carbon and nitrogen, via the microbial loop (Gilbert et al. 1998; Gilbert and Mitchell 2006; Thormann 2006).

Because living *Sphagnum* has specific morphological, physiological, and ecological properties and are associated with a specific microbial loop, we proposed to use the original term "sphagnosphere" to represent such microecosystem. Thus, we define the "sphagnosphere" as a specific microecosystem between living S*phagnum* and its associated microorganisms. "Sphagnosphere" represents the first scale in peatlands allowing determining any relation with its surrounding environment.

3.4.1 Effect of Sphagnum Extract on Sphagnum Associated MicroOrganisms

The dynamic of *Sphagnum* associated microorganisms is usually explained by physical and chemical factors (e.g. water level, pH, redox potential). Unfortunately, the allelochemical interactions between *Sphagnum* and its associated microbial communities are rarely studied. Recently, Jassey et al. (2011a, b) suggested that phenolic compounds were involved in the structure of microbial assemblages in *Sphagnum* peatlands, especially on testate amoebae communities. Testate amoebae are abundant and diverse-shelled protozoa living in a wide range of habitats from soils to peatlands (Mitchell et al. 2008). They are an important group in microbial food web because they feed on a wide range of prey such as bacteria, algae, fungi, rotifers, nematodes, or other small testate amoebae. Owing to their role in microbial food web, they play a crucial role in the microbial loop and nutrient cycling (Gilbert et al. 2003; Wilkinson and Mitchell 2010). In *Sphagnum* peatlands, testate amoebae communities were strongly correlated to surface wetness conditions and water chemistry (Mitchell et al. 2008). Jassey et al. (2011a) demonstrated for the first time that phenolics explained a variance partitioning of 25.6% as physical factors (34.1%) and as chemical factors (16.8%) of their community composition along *Sphagnum* shoots. It strongly suggested the potential role of these compounds in their autecology. More precisely, a relationship between testate amoebae and polyphenols was confirmed. For example, the density of *Euglypha strigosa* was significantly correlated to the concentrations of *Sphagnum* water-soluble phenolics (Fig. 3.6). Although such result does not allow a conclusion to be strongly drawn on a possible direct positive effect

Fig. 3.6 *Euglypha strigosa*
density (ind.g^{-1} DM) plotted
against the concentrations of
S. fallax water-soluble
phenolics (mg g^{-1} DM)
(ANOVA test, $P = 0.02$).
E. Strigosa photo is
reproduced, with kindly
permission of E.A.D.
Mitchell (adapted from
Jassey et al. 2011c)
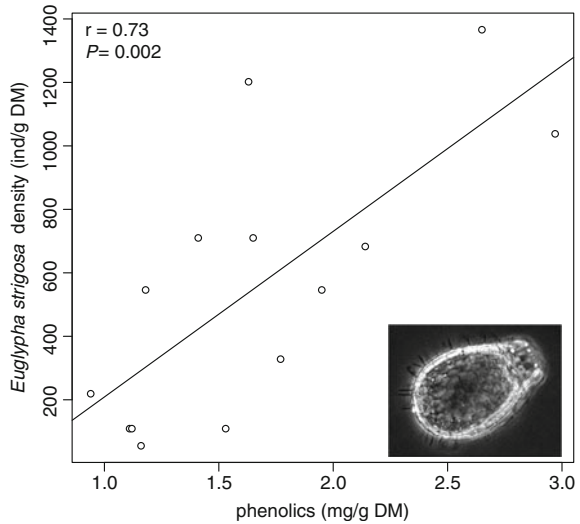
(or indirect effect on the food web) of phenol released by *Sphagnum* on *E. strigosa*,
it raises the issue of the possible role of phenolics on microbial communities.

3.4.2 Effect of Sphagnum Extract on Germination and Early Growth of Vascular Plants

The bulk of research on allelopathy focuses on the visible effects of allelochem-
icals (secondary effects) particularly on germination and growth. These effects are
tested with plant extract when the compounds have not been yet identified as it is
the case for *Sphagnum* (Chiapusio et al. 1997).

The experimental procedure involved testing the effect of *S. fallax* extract on
the germination and early growth of three vascular species a monocot *Lolium
perenne,* a dicot *Raphanus sativa*, and a local gymnosperm peatland species *Pinus
uncinata*. Extracts of *S. fallax* were realized by pressing by hands *Sphagnum*
plants. The obtained extract was (1) directly used in the germination experiments
and corresponded to the 100 % extract and (2) diluted in distilled water to obtain a
75 % extract. The pH of each *Sphagnum* extract was measured and other controls
were also realized with the same pH. As no statistical difference between the
"normal control" and the "buffered pH" was found, we concluded that the pH had
no effect on the germination process. A 100 % *Sphagnum* extract and the 75 %
extract had a drastic negative effect on *Lolium* germination (7- and 4-times,
respectively less than the control) but also on *Pinus* germination (two times less
than the control for both treatments) (Fig. 3.7a). On the contrary, no effect on
germination of radish seeds was observed. Differences between monocot and dicot
could explain such germination differences but need complementary experiments.

Fig. 3.7 Effect of *S. fallax* extract on **A** the germination (%) of *Lolium perenne*, *Raphanus sativus*, and *Pinus uncinata*. Mean ± S.D. (*n* = 5). (Mann–Whitney test, *P* < 0.05) and on **B** radicle length (mm) of *L. perenne*, *R. sativus,* and *P. uncinata*. Mean ± S.D. (*n* = 5). (Mann–Whitney test, *P* < 0.05) (Unpublished data)
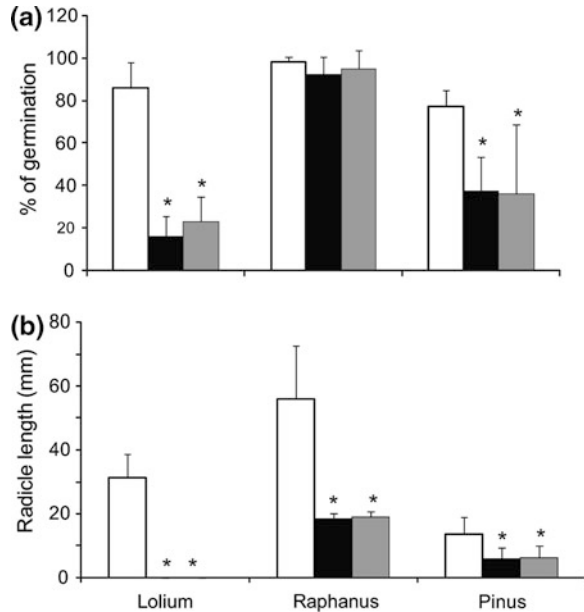
Sphagnum extract on the radicle growth was drastic for all tested species (Fig. 3.7b). Indeed, inhibition of root was noticed for radish (30 %) and for *Pinus* (40 %) for both treatments. Moreover, when the *Lolium* seeds germinate they were then not able to develop their radicle in both *Sphagnum* extracts. The radicle growth was totally suppressed.

Living *Sphagnum* extracts offered interesting perspectives for allelochemical interactions in the functioning of peatlands to understand vegetation dynamics. Similar results, but with other bryophytic genus, were described (Zamfir 2000). Indeed, the homogeneous mat of *Sphagnum* is known to be due to low nutrient availability, anoxia, low temperatures, and high acidity (Van Breemen 1995). Along our results, *Sphagnum* ability to compete other plant species, especially vascular plants, could also be due to allelochemical production. The term interference, combining competition, and allelopathy becomes relevant then.

Moreover, the allelochemical *Sphagnum* extract could also be a good candidate as biopesticides (Chiapusio et al. 2005). Observed inhibitions of germination and growth are promising comparing with other allelochemicals (e.g. 2-benzoxazolinone, Chiapusio et al. 2004; Hussain et al. 2008). Such results constitute a crucial step for further experiments.

3.5 Conclusions

Water-soluble phenolics produced by living *Sphagnum* were found to be of great interest for the functioning of peatlands. First, they were concentrated in the top layer of Sphagnum and their concentrations varied along the seasons. *Sphagnum* peroxidases

constituted the main oxidative system and fungal-phenoloxidases were proposed to be inhibited by phenolics. In such context, the polyphenolic phenoloxidase interplay is especially crucial to understand the mechanisms by which peatlands react under a climatic change. Indeed, peatlands could become weaker sinks of carbon but depends on their proper ecological conditions (e.g. ecological gradient).

Second, our hypothesis of allelopathic interactions in peatlands made sense with the evidence of original interactions between *Sphagnum* phenolics and its associated microorganisms, the "sphagnosphere." Of course, allelopathy was not considered as the only factor explaining microbial dynamic and microbial food chain but was revealed to be fully considered and not neglected. Moreover, the drastic inhibition/delay of *Sphagnum* extract on germination and radicle growth of vascular plants could also explain the *Sphagnum* dominance under surrounding plants.

We still have a lot to learn from bryophyte secondary metabolite, not only to better understand their ecological role but also to discover original natural products that could be used as natural biopesticide. However, research is still needed on the identification and the biodegradability of bryophyte allelochemicals, which are largely unknown until now.

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