ECOLOGY LETTERS

Ecology Letters, (2016) doi: 10.1111/ele.12714

REVIEW AND SYNTHESIS

Mixotrophy everywhere on land and in water: the *grand écart* hypothesis

Marc-André Selosse,[1,2]*
Marie Charpin[3] **and Fabrice Not**[4]

Abstract

There is increasing awareness that many terrestrial and aquatic organisms are not strictly heterotrophic or autotrophic but rather mixotrophic. Mixotrophy is an intermediate nutritional strategy, merging autotrophy and heterotrophy to acquire organic carbon and/or other elements, mainly N, P or Fe. We show that both terrestrial and aquatic mixotrophs fall into three categories, namely necrotrophic (where autotrophs prey on other organisms), biotrophic (where heterotrophs gain autotrophy by symbiosis) and absorbotrophic (where autotrophs take up environmental organic molecules). Here we discuss their physiological and ecological relevance since mixotrophy is found in virtually every ecosystem and occurs across the whole eukaryotic phylogeny, suggesting an evolutionary pressure towards mixotrophy. Ecosystem dynamics tend to separate light from non-carbon nutrients (N and P resources): the biological pump and water stratification in aquatic ecosystems deplete non-carbon nutrients from the photic zone, while terrestrial plant successions create a canopy layer with light but devoid of non-carbon soil nutrients. In both aquatic and terrestrial environments organisms face a *grand écart* (dancer's splits, i.e., the need to reconcile two opposing needs) between optimal conditions for photosynthesis vs. gain of non-carbon elements. We suggest that mixotrophy allows adaptation of organisms to such ubiquist environmental gradients, ultimately explaining why mixotrophic strategies are widespread.

Keywords

Biological pump, carnivorous plants, hemiparasitism, kleptoplastidy, mycoheterotrophy, mycorrhiza, osmotrophy, phagotrophy, photosymbiosis, protists.

Ecology Letters (2016)

INTRODUCTION

Common knowledge and textbooks often contrast two exclusive trophic strategies for carbon acquisition by living organisms: heterotrophy vs. autotrophy. Autotrophs assimilate CO_2 through chemolithotrophy or photosynthesis to produce their own biomass using environmental energy sources, while heterotrophs acquire carbon from already existing biomass of surrounding organisms, alive or dead. Another epistemological reason supports this basic partition since macroscopic organisms, which are easily observable and often used as research models, superficially fit this duality. Most animals are strictly heterotrophic, while plants grow on mineral resources. Ecological echoes of this trophic segregation are to be found in trophic webs, in the opposition between primary producers, leading to primary biomass, and consumers, leading to secondary or higher level biomass.

Yet an intermediate strategy, mixotrophy, merges autotrophy and heterotrophy to acquire carbon and/or other elements, such as N, P or S (collectively called 'non-carbon' elements hereafter). Although mixotrophy received various definitions (Box 1), we consider here a broad physiological definition: the combination of autotrophic and heterotrophic nutrition within an organism, more precisely the physiological feature of an organism whose cells use both photosynthesis and external organic matter as a source of carbon and/or non-carbon elements. Three main modes of mixotrophy are identified (Box 1) and anciently recognised in some land plants and some unicellular algae. Accumulating evidence now shows that mixotrophy is pervasive in both aquatic and terrestrial ecosystems. In contrast to the increasing awareness for aquatic ecosystems (Flynn *et al.* 2013; Worden *et al.* 2015; Mitra *et al.* 2016), reports of mixotrophy in land plants remains limited to isolated studies on nutritional mechanisms that are rarely conceptually united as mixotrophic and are hardly analysed at the ecosystem level (Selosse & Roy 2009). In addition, research groups working on terrestrial and aquatic models are distinct and focus on different taxa and

---

[1] *Institut de Systématique, Évolution, Biodiversité (ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005 Paris, France*

[2] *Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland*

[3] *Université Blaise Pascal, Clermont-Ferrand, CNRS Laboratoire micro-organismes: Génome et Environnement, UMR 6023, 1 Impasse Amélie Murat, 63178 Aubière, France*

[4] *Sorbonne Universités, UPMC Université Paris 06, CNRS, Laboratoire Adaptation et Diversité en Milieu Marin UMR7144, Station Biologique de Roscoff, 29680, Roscoff, France*

**Correspondence: E-mail: ma.selosse@wanadoo.fr*

Box 1 A definition and three main mechanisms for mixotrophy.

We define mixotrophy as the physiological feature of an organism whose cells (at least some cells, for multicellular organisms) use both photosynthesis and external organic matter as a source of carbon and/or non-carbon elements. Thus, we do not consider as mixotrophic an autotrophic organism with heterotrophic cells using organic matter of internal origin. Our definition does include endosymbiotic consortia where photosynthetic cells are embedded in an otherwise heterotrophic cell, which also uses external organic matter. Three main mechanisms allow mixotrophy under our definition:

Absorbotrophic (=osmotrophic) mixotrophy: uptake of soluble organic matter found in the environment, e.g., low-molecular-weight molecules (Schmidt *et al.* 2013). The word 'osmotrophic' ('osmomixotrophy' in Schmidt *et al.* 2013) insidiously suggests that osmotic forces account for this process, while active uptake is involved, so that we prefer the term 'absorbotrophic'. Low-molecular-weight molecules are targeted, but some enzymatic processing, outside of or inside the cell after endocytosis, can be involved. Absorbotrophy occurs in multicellular organisms, such as land plants and in virtually all algae. Because of this, some aquatic research scientists tend in practice to restrict the term mixotrophy to the combination of the next two strategies (Flynn *et al.* 2013), but we and others (e.g. Stoecker 1998) do not follow this restrictive definition. With the exception of a few microalgae, the physiological and ecological relevance of absorbotrophic mixotrophy remains poorly quantified.

Biotrophic (= symbiotic) mixotrophy: uptake of organic matter derived from interactions with other living organisms ('non-constitutive mixotrophs' *sensu* Mitra *et al.* 2016). Such a relationship does not kill the organisms involved, but range from parasitic to mutualistic. This strategy is widespread in terrestrial ecosystems, with green plants that are hemiparasitic (i.e. parasitise other living plants) or partially mycoheterotrophic (i.e. obtain organic matter from mycorrhizal fungi); some plants even use the wastes of symbiotic insects. Biotrophic mixotrophy is also common in aquatic ecosystems where some protists or metazoans live in obligatory symbioses with photosynthetic microalgae (photosymbioses). In all cases, biotrophic mixotrophy appears as a secondarily evolved ability, by autotrophs in terrestrial ecosystems and by heterotrophs in aquatic ecosystems.

Necrotrophic (= predatory) mixotrophy: uptake of organic matter by predation on other living organisms, entailing their death, also called 'phagomixotrophy for planktonic protists' (Schmidt *et al.* 2013) or 'constitutive mixotrophs' (Mitra *et al.* 2016). Frequently observed in microalgae (by phagocytosis and digestion of other cells), this strategy is represented in land plants by carnivorous species (predating on small animals or unicellular organisms). Although the latter are secondarily evolved, phagocytotic abilities in microalgae is often a plesiomorphic trait, derived from the phagocytotic abilities of their heterotrophic ancestors, which allowed them to engulf the precursors of plastids. Aquatic protists or metazoans that retain plastids captured from autotrophs (kleptoplastidy) are considered as necrotrophic mixotrophs, since these plastids never escape the host cell.

Comments on other more restrictive definitions. In the past, this diversity of mechanisms has somewhat blurred the uniting feature, namely the combination of autotrophy and heterotrophy within a single organism. Several authors use more restrictive definitions. First, some aquatic ecologists only consider necrotrophic mixotrophy (e.g. Flynn *et al.* 2013): one reason is to limit mixotrophy to cases where there is a predation and to avoid symbiotic mechanisms; yet, other researchers include biotrophic mixotrophy (e.g. Schmidt *et al.* 2013; Mitra *et al.* 2016). Second, some terrestrial ecologists consider mixotrophy as a too vague term, e.g., for mycorrhizal associations (see Hynson *et al.* 2013): they prefer more precise terms (hemiparasitism, partial mycoheterotrophy, etc.). This limited in the past the emergence of common questions and approaches on these strategies (but see Selosse & Roy, 2009 and Schmidt *et al.* 2013). Third, the universality of absorbotrophy sometimes lead to excluding it from mixotrophy: however, many authors encompass it in a large view of mixotrophy (e.g. Heifetz *et al.* 2000; Schmidt *et al.* 2013).

We do not criticise more restricted definitions, when explicit, in other frameworks. For the present review, we think that the organismal feature underlined by our broad definition is heuristic because it addresses a common physiological fact (combination of autotrophy and heterotrophy within a single organism) beyond (1) mechanisms and (2) fitness impact(s) on the partners, if any. Finally, any more restrictive definition would not annihilate our central question (why is mixotrophy everywhere?), because any of the three mechanisms presented above are present in both terrestrial and aquatic ecosystems.

ecosystems, so that a few exceptions apart (e.g. Schmidt *et al.* 2013) the ubiquity of mixotrophy in the biosphere is overlooked. Moreover, the obvious differences between aquatic and terrestrial environments often lead to underestimation of common traits and questions: indeed, mixotrophy is one of these, suggesting that beyond differences, some similar mechanisms and constraints exist. In this context, a general conceptual framework is required to understand mixotrophy across eukaryotic lineages and ecosystems.

Here we review the evidence of phylogenetic and ecological spectra of mixotrophy in aquatic and terrestrial ecosystems, with a particular emphasis on poorly investigated terrestrial mixotrophs. We highlight how diverse physiological mechanisms achieve mixotrophy and their respective ecological relevance. Considering that mixotrophy is everywhere, we then propose a unifying hypothesis explaining why mixotrophy evolved in aquatic and terrestrial ecosystems, and suggesting that ecosystem dynamics lead to recurrent and ubiquitous selection of mixotrophy.

MIXOTROPHY IN TERRESTRIAL ECOSYSTEMS

Land plants (the so-called Embryophyta; Fig. 1), considered as primarily autotrophic, can also acquire organic carbon by diverse pathways (partially reviewed in Selosse & Roy 2009; and Schmidt *et al.* 2013), with sources ranging from living

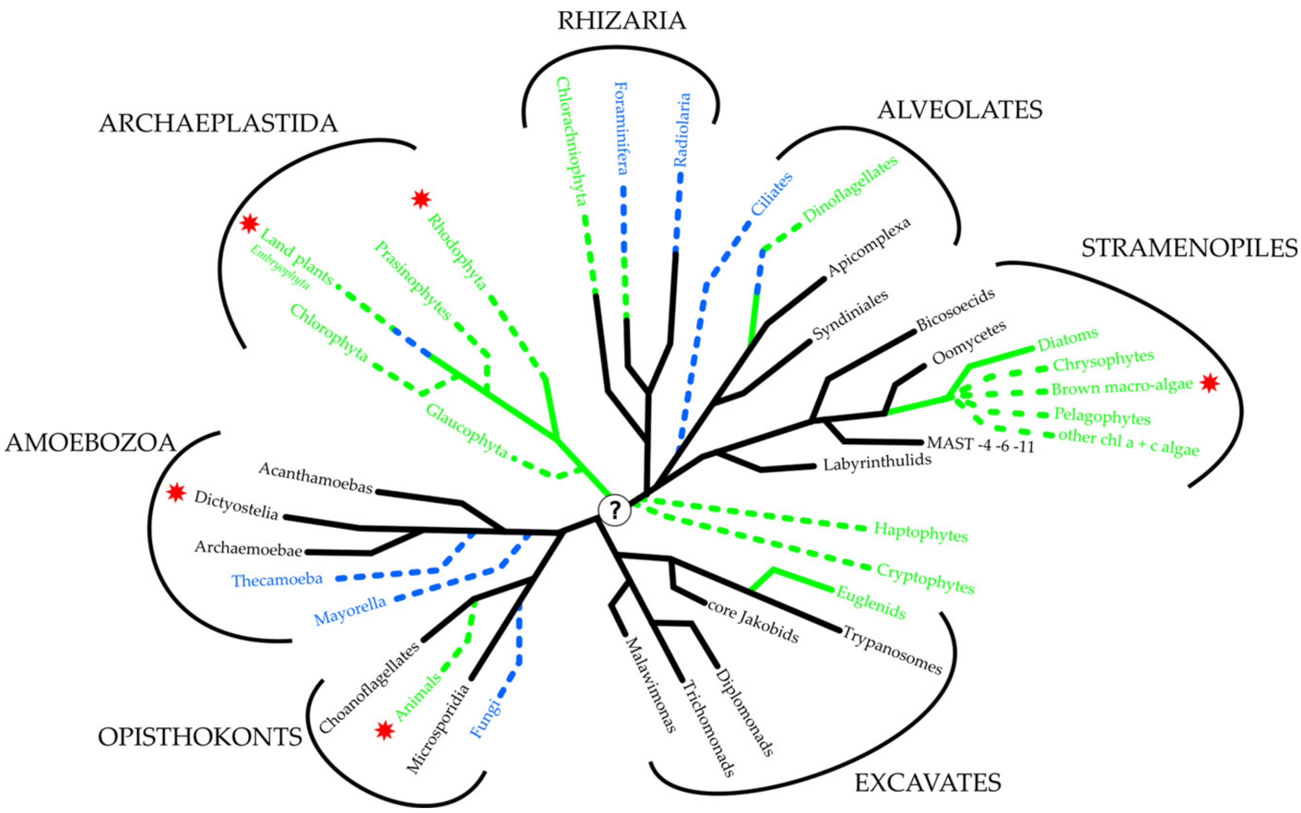

Figure 1 Mixotrophy is everywhere in the eukaryotic phylogeny (phylogenetic tree representing major eukaryotic lineages adapted from Baldauf 2008). Red stars indicate lineages for which multicellular organisms exist; all others are composed of unicellular eukaryotes only. Strictly autotrophic and heterotrophic taxa are represented in green and black respectively. Dashed green represents taxa able to perform absorbotrophic and necrotrophic mixotrophy. Dashed blue represents taxa able to perform biotrophic mixotrophy. Whenever the two colours are displayed, it indicates that all mixotrophic strategies can coexist in the lineage.

organisms (animals, plants or microbes), which are prey or symbiotic partners (necrotrophic or biotrophic mixotrophy respectively; Box 1), to dead organic matter by way of absorbotrophic mixotrophy. Here, we describe the extent to which these pathways provide resources and their relevance at the ecosystem level.

Necrotrophic mixotrophy in carnivorous and animal-associated plants

Carnivory evolved up to six times in land plants (Ellison & Gotelli 2009; Fig. 2). Strict carnivorous plants have both traps (e.g. passive sticky leaves, active trapping designs or pitchers) and secretion of enzymes to digest prey whose non-carbon elements compensate for nutrient-poor environments, such as epiphytic niches and alpine or boreal soils (Ellison & Gotelli 2009). They may also gain carbon from prey (Adamec 1997), and ^{14}C-labelled insects on *Drosera* spp. revealed acquisition of 47–80% of carbon from insect sources (Ashley & Gennaro 1971; Dixon *et al.* 1980). Such labelling methods often underestimate such acquisition as they neglect (1) carbon lost by respiration after assimilation and (2) photosynthetic refixation of CO_2 emanating from decaying insects (but this pathway may make a limited contribution; Ashley & Gennaro 1971). Carbon is at least transferred by way of the nitrogen-containing molecules recovered from the prey, such as urea and amino acids, thus hitchhiking with nitrogen (Rischer *et al.* 2002; Karagatzides *et al.* 2009). Uptake of carbohydrates has not been demonstrated formally, but the endocytosis of prey macromolecules in some carnivorous plants (Adlassnig *et al.* 2012) opens the way to non-selective uptake of various molecules. Further enlarging the spectrum of plants that use necrotrophic mixotrophy, *Arabidopsis* and tomato (*Solanum lycopersicum*) root cells can incorporate and digest unicellular soil microbes (Paungfoo-Lonhienne *et al.* 2010), although this awaits generalisation to other models.

While most carnivorous strategies are necrotrophic, in some cases they also involve symbionts that help access prey resources, and thus encompass biotrophic mixotrophy when considering the relationship with these symbionts. A diffuse carnivory without enzymes ('protocarnivory') exists, for example, in plants that catch animals by sticky hairs: microbial breakdown of large molecules from glued prey may supplement nutrition of plants from more than 26 families (Chase *et al.* 2009). Although its exact nutritional relevance remains unknown, this microbial carnivory may have allowed the repeated evolution of true carnivorous plants, i.e., which produce digestive enzymes themselves, especially in nutrient-poor environments. As an extreme case, the sticky *Roridula* species associates with symbiotic Hemiptera that eat glued prey deliver carbon and non-carbon elements to the plant through faeces (Anderson 2005). Plants associated with ants, especially

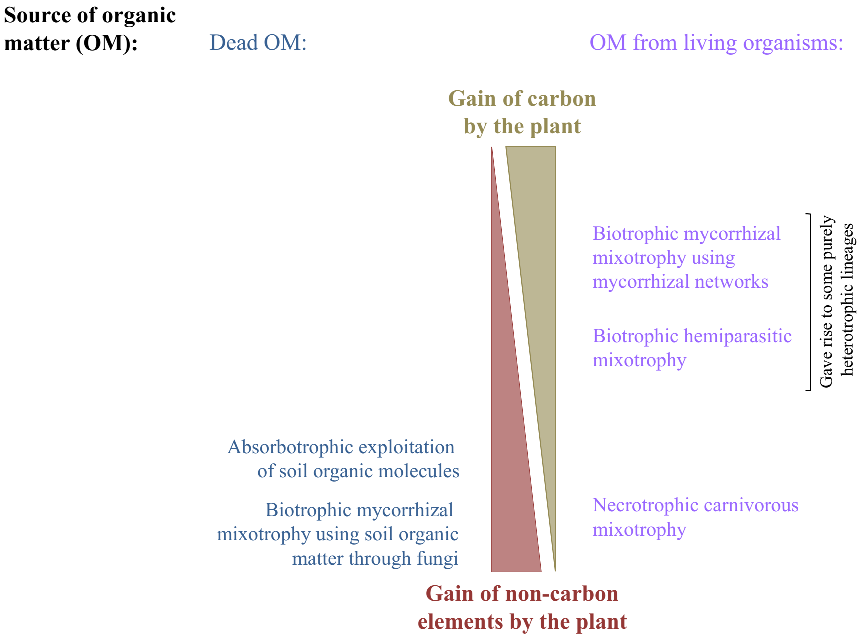

Figure 2 Diversity of the mixotrophic strategies in land plants: they use (from top to bottom) other plants (either directly or by way of shared fungi forming a common mycorrhizal network, grey area), soil dead organic matter (either directly or by way of mycorrhizal fungi) or even animal prey.

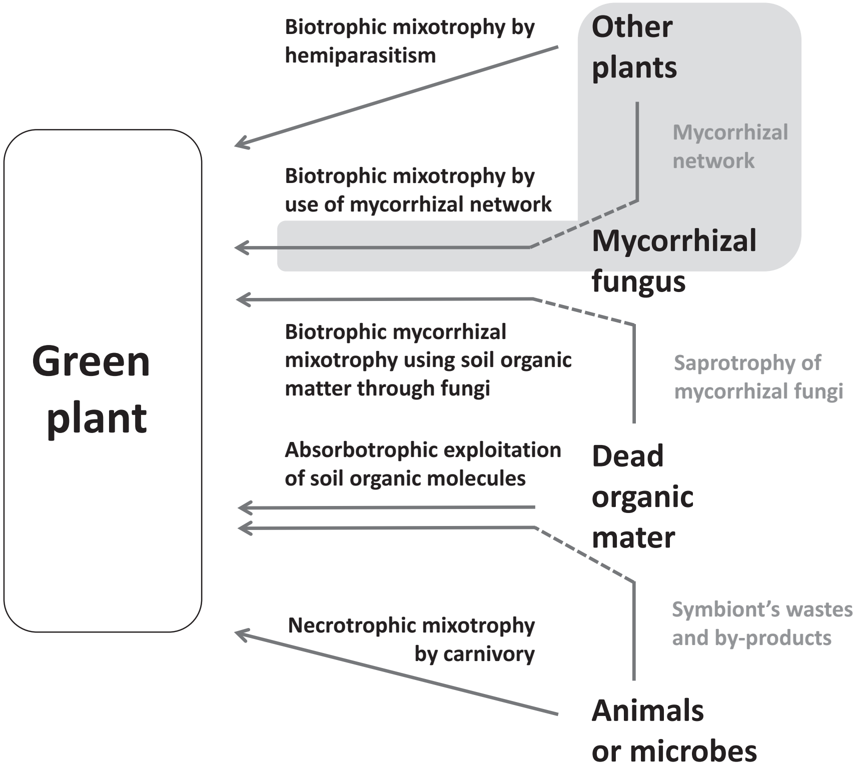

Figure 3 Relative relevance for acquisition of carbon and non-carbon elements of the diverse mixotrophic strategies of land plants.

epiphytic ones, gain non-carbon elements and perhaps carbon from ant waste, faeces and cadavers deposited in the refuges inhabited by the ants (the so-called domatias; Treseder *et al.* 1995), and these resources are derived from prey and food harvested out of domatias. In numerous unrelated ant–plant symbioses, ants tend fungi in domatia, which are involved in the cycling of nitrogen, at least, from ant waste to the plant (Defossez *et al.* 2009 Defossez *et al.* 2011). Finally, indirect predation and nutrient transfer to plants can be assisted by fungi alone, as demonstrated for *Metarhizium*, a fungus living in plant tissues and also in soil where it kills insects (Behie *et al.* 2012). The mixotrophy associated with such endophytic fungi, which is reminiscent of that based on mycorrhizal fungi (see Biotrophic mixotrophy based on mycorrhizal fungi), had hitherto been overlooked.

Gain of carbon is probably not a main advantage of proto- and true carnivory, because no fully heterotrophic plant relies solely on this carbon acquisition. The carbon gain is probably insufficient because prey are smaller than traps, and the *raison d'être* is thus mainly linked to non-carbon elements (Fig. 3) and indeed traps are often lost when these elements, especially nitrogen, abound (Ellison & Gotelli 2002). Carbon supplementation by prey may, however, be relevant in some environments that are shaded (where some carnivorous plants grow because insects are locally abundant) or aquatic (where water entails CO_2 limitations; Adamec 2006). Finally, no study has addressed how carnivory affects communities and ecosystems: we can only speculate about local impact on prey species and on trophic webs; being richer in non-carbon elements than their oligotrophic environments, carnivorous plants may locally enhance the pools and turnover of these elements.

Biotrophic mixotrophy in plant–parasitic plants

Hemiparasitism evolved at least five times in land plants (Westwood *et al.* 2010). Hemiparasitic plants are photosynthetic, sometimes with reduced photosynthetic abilities, and obtain their non-carbon elements by parasitising other plants (Fig. 2), including economically important crops. Their modified roots (called haustoria) connect them to host's roots or stems, and derive the sap from xylem either through their elevated transpiration rate or through active capture by specialised transfer cells (Těšitel 2016). Thanks to efficient derivation of non-carbon elements, hemiparasites are successful in nutrient-poor environments (Quested 2008), including the epiphytic niche for mistletoes (Loranthaceae).

Hemiparasites also recover organic carbon from their host, despite a low concentration of carbon in xylem (Fig. 3). Beyond direct evidence based on transfer of radiolabelled carbon, host carbon contribution to hemiparasite biomass can be estimated from natural abundance of ^{13}C isotope (Box 2). Těšitel *et al.* (2010, and references therein) reported that the host provides 20–80% of hemiparasite biomass in the Olacaceae and Orobanchaceae root hemiparasites, and 50–80% in the Loranthaceae stem hemiparasites. Three other features support a host contribution to carbon acquisition in hemiparasites. First, some hemiparasites (e.g. Orobanchaceae such as *Striga* spp.) initially grow underground on host roots, without photosynthesis (Westwood *et al.* 2010). Second, achlorophyllous variants can survive (Press *et al.* 1991). Finally, fully heterotrophic species, the so-called holoparasites, evolved three times from hemiparasitic ancestors within Orobanchaceae (McNeal *et al.* 2013) and twice within Cuscutaceae

Box 2 Isotopic (^{13}C) tools to study and quantitatively assess mixotrophy in terrestrial environments

Whenever heterotrophic and photosynthetic sources have different ^{13}C enrichment, the ^{13}C abundance in mixotrophic biomass is intermediate between these two sources. Mixotrophic tissue adds a biomass portion x with heterotrophic ^{13}C abundance and (1-x) with autotrophic ^{13}C abundance. When heterotrophic and autotrophic references are available for both sources, the observed ^{13}C abundance in mixotrophic tissues allows estimation of x using a linear two-source mixing model (Gebauer & Meyer 2003).

Hemiparasitic mixotrophs have C3 photosynthesis, so that such calculations can easily be done if they parasitise hosts with C4 or CAM photosynthesis that have higher ^{13}C abundance due a different carbon fixation pathway (Těšitel *et al.* 2010): abundances expected for C3 plants and measured for C4 hosts can be used as references. Whenever hemiparasites parasitise C3 hosts, the calculation can be done if there are sufficient physiological differences between the host and the hemiparasite. The latter has wide open stomata to derive the host's xylem sap and a lower photosynthetic efficiency (see main text), which increase the fractionation against ^{13}C as compared with the host (Farquhar *et al.* 1989). Thus, the expected ^{13}C abundance in its own photosynthesis can be estimated from physiological models (e.g. Wang *et al.* 2008).

Mixotrophs using mycorrhizal networks (Fig. 2) are also C3 plants. The heterotrophy level x can be estimated whenever they associate with ectomycorrhizal Asco- and Basidiomycota, whose biomass is enriched in ^{13}C (Trudell *et al.* 2003). Indeed, such fully mycoheterotrophic plants (Gebauer & Meyer 2003) or achlorophyllous variants in mixotrophic populations (Julou *et al.* 2005; Abadie *et al.* 2006) are enriched in ^{13}C and can be used as a reference for heterotrophic biomass. Unfortunately, while Glomeromycota are the most frequent mycorrhizal fungi, demonstration and quantification of mixotrophy in candidate plants is limited by the fact that they do not differ isotopically from autotrophic plants (Courty *et al.* 2011; Courty *et al.* 2014), so that isotopic content has so far remained silent as to the most frequent mycorrhizal networks.

The use of natural ^{13}C abundance in mixotrophs reflects the contribution of auto- and heterotrophic nutrition, integrated over the whole life of an organ. Yet, there are several caveats. First, catabolism is neglected (only one study of respiratory CO_2 in the mixotrophic orchid *Cephalanthera damasonium* suggested that x does not differ between catabolism and anabolism; Roy *et al.* 2013). The heterotrophy level x varies among organs and among developmental stages (Pageau *et al.* 1998; Roy *et al.* 2013; Gonneau *et al.* 2015), so that the 'heterotrophic level' x is not a fixed value for a given species or individual. Finally, other factors (such as content in lignocellulose or reserves) affect ^{13}C abundance (Cernusak *et al.* 2009), so that the x values should be viewed with caution, and at best comparatively between samples from the same site or plant.

(Braukmann *et al.* 2013): they rely on host carbon thanks to additional connections to the sugar-rich sap from phloem (Těšitel 2016).

Hemiparasites have a high impact on ecosystem structure and functioning (Press & Phoenix 2005; Watson 2009; Bell & Adams 2011). They have sublethal effects on their hosts, which grow less well, and thus reduce primary production (Borowicz & Armstrong 2012). Whenever they attack an abundant host species, they liberate space for subordinate ones and increase plant biodiversity; conversely, an opposite effect is achieved if they attack a subordinate species. Globally, by modulating community composition, they have a keystone species role in ecosystems (Press & Phoenix 2005; Watson 2009). Hemiparasites also support their own trophic webs, including parasites, fruit and pollen dispersers (Press & Phoenix 2005). Finally, their high content in non-carbon elements robbed from their host makes them more palatable and more easily degraded in soil, so that they can locally modify biogeochemical cycles, enhancing turnover of nitrogen and phosphorus availability (Quested 2008; Watson 2009), likely mitigating their direct impact on primary biomass.

Biotrophic mixotrophy based on mycorrhizal fungi

Most land plants that associate with soil fungi form a dual organ, the mycorrhizae, where the fungus usually exchanges water and non-carbon elements collected in soil for plant photosynthates (van der Heijden *et al.* 2015). These fungi have evolved many times independently: while Glomeromycota form the ancestral mycorrhizal type that persists in most plants, several Asco- and Basidiomycota later replaced them in some plant lineages, e.g., forming the so-called ectomycorrhizae and ericoid mycorrhizae (van der Heijden *et al.* 2015). Mycorrhizal fungi potentially allow mixotrophy based on two organic matter sources, namely soil dead organic matter and surrounding plants sharing the same fungi (Fig. 2).

Mixotrophy by use of soil organic matter through fungi

A debated question is whether mycorrhizal fungi can access soil organic matter and provide it to the plant. Genomic evidence shows that mycorrhizal fungi have very limited genetic abilities to exploit complex carbon substrates (Floudas *et al.* 2012; Kohler *et al.* 2015). Yet ectomycorrhizal fungi have retained some enzymatic ability from their saprobic ancestors, which they mainly use to acquire organic nitrogen and phosphorus (Floudas *et al.* 2012; Rineau *et al.* 2013). Accordingly, application of ^{13}C- or ^{14}C-labelled litter contributes to limited labelling of ectomycorrhizal fungi (< 2% in the following year; Treseder *et al.* 2006) and of host roots (< 1% over 6 months; Bréda *et al.* 2013); although such values include refixation of respiratory CO_2 by anaplerotic reactions in root cells. A particular mycorrhizal type, called ericoid mycorrhiza because it evolved once in an Ericaceae clade (Lallemand *et al.* 2016), accesses organic nitrogen and phosphorus thanks to Asco- and Basidiomycota fungi with a high degrading ability (Kohler *et al.* 2015). This process allowed adaptation to poorly mineralised, high latitude or high altitude soils where ericoid mycorrhizal Ericaceae abound (Read & Kerley 1995; van der Heijden *et al.* 2015).

Such mycorrhizal associations are more relevant in terms of non-carbon elements than C uptake (Fig. 3). By bypassing the mineralization step, they avoid soil oligotrophy through faster removal of organic nutrients, inducing a positive feedback towards a mycorrhizal mixotrophic strategy. A modelling approach (Orwin *et al.* 2011) further suggests that, under nutrient-limited conditions, organic nutrient uptake by mycorrhizal fungi increases plant carbon fixation and, as a result, inputs carbon to soil through mycorrhizal fungi: the increased ratio of carbon to non-carbon elements in soil finally decreases decomposition, and therefore, nutrient recycling. This in turn favours plants accessing non-carbon elements in organic form: this positive feedback could account for the domination of ectomycorrhizal associations (Kuyper & Kiers 2014) or ericoid mycorrhizal associations (Read & Kerley 1995) in some ecosystems, such as boreal or alpine ones, due to a reshaping of nutrient cycling and biodiversity.

Mixotrophy by use of mycorrhizal networks

Most mycorrhizal fungi exhibit a low specificity and a given fungus can associate with several plant species (Selosse *et al.* 2006; Simard *et al.* 2012). Some mixotrophic species use the resulting mycorrhizal networks to recover carbon in a strategy called mycoheterotrophy (Selosse & Roy 2009; Hynson *et al.* 2013), which reverses the mycorrhizal carbon flow: although the usual and ancestral direction is from plant to fungus, mycoheterotrophic plants recover fungal carbon. While some of these plant species are achlorophyllous and fully mycoheterotrophic, others are green and mixotrophic because they combine photosynthesis and partial mycoheterotrophy. The combination is successive in development since after initial mycoheterotrophy, adults become autotrophic. These plants have very small seeds or spores, devoid of reserves, and rely on fungi for subterranean germination thanks to mycoheterotrophy (Eriksson & Kainulainen 2011). When seedlings turn green, they start providing carbon to the fungus (Hynson *et al.* 2013; Field *et al.* 2015), although they remain mixotrophic in some species (see below).

Mycoheterotrophic germination occurs in diverse ferns and lycopods (Field *et al.* 2015), in Burmaniaceae (Bolin *et al.* 2016) and in some Ericaceae (Hashimoto *et al.* 2012): they target fungi that are mycorrhizal on surrounding plants and thus indirectly exploit surrounding plants (Fig. 2). This also happens in all orchids, in which the origin of fungal resources remains debated: some fungi are mycorrhizal on non-orchid plants, but others (the so-called 'rhizoctonias') are considered saprobic (Dearnaley *et al.* 2012). Indeed, the genomes of rhizoctonias display a huge set of lytic enzymes (Kohler *et al.* 2015), but increasing evidence shows that they grow as endophytes in the roots of non-orchid plants (Selosse & Martos 2014), so that a large part of their carbon resources may be of plant origin, as for true mycorrhizal fungi.

In some species adults are photosynthetic but remain partly mycoheterotrophic, as described from at least six independent orchid lineages (Hynson *et al.* 2013) and most pyroloids (Tedersoo *et al.* 2007) that associate with fungi ectomycorrhizal on nearby trees (Selosse *et al.* 2016). Four lines of evidence support their partial mycoheterotrophy. First, their photosynthesis displays limitations, either intrinsically

(Girlanda *et al.* 2006) or due to shaded environments (Julou *et al.* 2005), and indeed, mixotrophy is an adaptation to living in shaded forest environments (Bidartondo *et al.* 2004). Second, achlorophyllous (= albino) variants, devoid of photosynthesis, sometimes survive (Julou *et al.* 2005; Roy *et al.* 2013). Third, the natural ^{13}C abundance is higher in mixotrophs than in autotrophic plants (Gebauer & Meyer 2003; Hynson *et al.* 2013), as a result of incorporation of ectomycorrhizal fungal biomass that is enriched in ^{13}C (Box 2). Finally, as mentioned above, phylogenetically close species are fully mycoheterotrophic (Barrett *et al.* 2014; Lallemand *et al.* 2016; Selosse *et al.* 2016).

An estimation of the frequency of mixotrophy based on mycorrhizal networks is limited by the fact that more than 80% of plant species are mycorrhizal with Glomeromycota that do not greatly differ in ^{13}C abundance from autotrophic plants (Courty *et al.* 2015), which hitherto has limited the detection of mixotrophy by natural isotopic abundance (Box 2). In this context, labelling experiments can demonstrate mixotrophy (Bolin *et al.* 2016), but do not quantify the contribution of received carbon to the global receiver's carbon budget. Thus, assessment of mixotrophy in Glomeromycota-associated photosynthetic plants remains largely pending, and since it is suspected to occur in several groups (Selosse & Roy 2009; Merckx *et al.* 2010), we call for more research in this direction.

Moreover, to our knowledge, the ecological relevance of mixotrophy based on mycorrhizal networks has never been directly and formally estimated. The nature (positive or negative) and intensity of their impact on fungal communities and on surrounding plant communities still has to be assessed. Interestingly, mixotrophs are sometimes enriched in nitrogen (Hynson *et al.* 2013; but data are currently lacking for other non-carbon elements) and thus more palatable (Roy *et al.* 2013): this may enhance nutrient turnover and locally modify biogeochemical cycles, as demonstrated for hemiparasitic mixotrophs (see above), especially in oligotrophic boreal forests where they abound (Tedersoo *et al.* 2007).

Absorbotrophic mixotrophy in terrestrial ecosystems

Beyond living biomasses exploited by previous strategies, low-molecular-weight organic matter also exists in soil, especially in soils with low turnover where non-carbon elements are often limiting. Plants exploit at least organic nitrogen resources, especially amino acids for which they have high-affinity transport systems (Näsholm *et al.* 2009; Schmidt *et al.* 2013). Beyond a simple absorbotrophy, a role of secreted proteases and even of endocytosis is suspected (Paungfoo-Lonhienne *et al.* 2008). We still do not know how organic nitrogen uptake contributes to the total needs of plants, how it is distributed among plant lineages and what is the ecological impact of such uptake (Näsholm *et al.* 2009). As for carnivorous plants, the carbon gained from absorbotrophy appears limited as compared with the plant's needs (Fig. 3) and although the possibility of similar uptake of small carbon molecules remains open, no fully known autotrophic plant relies on this carbon source only. In environmental conditions, microbes likely compete efficiently with plants in acquiring low-molecular-weight organic nutrients (Rousk *et al.* 2014). In a way reminiscent with some types of carnivory (see Necrotrophic mixotrophy in carnivorous and animal-associated plants), an association with microbes can be relevant for accessing environmental dead organic matter: for instance, plants that form aerial holes or pouches (the phytotelmata) where dust and rainwater accumulate, e.g., epiphytic Bromeliaeace, gain nutrients from microbial breakdown in phytotelmata (Nishi *et al.* 2013).

Summary

With the exception of organic matter uptake from soil, which may be ancestral but remains of debatable importance, mixotrophy in land plants is an evolutionarily derived condition, mainly achieved by interaction with other organisms. Terrestrial mixotrophy evolved many times from primarily autotrophic ancestors and never from heterotrophic ancestors in the examples above. Beyond a role for non-carbon elements that make mixotrophy adapted to oligotrophic environments, variable carbon gain is achieved (Fig. 3) that can even permit adaptation to shaded environments. In spite of many physiological studies, few investigations so far have incorporated mixotrophic strategies at the ecosystem and nutrient cycling levels, although this potentially modifies the environment. Well described as botanical curiosities nearly 150 years ago after Darwin (1875) popularised them, terrestrial mixotrophs are now better described from a physiological viewpoint, but hardly enter the field of terrestrial ecology.

MIXOTROPHY IN AQUATIC ECOSYSTEMS

Besides large metazoans, seagrasses and macroalgae, aquatic life is essentially microscopic and belongs to unicellular groups, often planktonic. This encompasses both bacteria (such as cyanobacteria) and eukaryotic unicellular organisms (i.e. protists) distributed throughout the entire eukaryotic tree of life. Protists include a number of unicellular species with plastids (commonly called microalgae; Fig. 1) that are major primary producers in aquatic ecosystems (Not *et al.* 2012). Yet most eukaryotic lineages encompass various trophic modes (Worden *et al.* 2015; Fig. 1), including the three main types of mixotrophy (Box 1). First, most microalgal lineages are able to carry out phagotrophy (i.e. necrotrophic mixotrophy), ingesting then digesting other cells. A variant uses plastids captured from surrounding autotrophic organisms: this so-called kleptoplastidy behaviour is common, but its impact is largely underestimated in aquatic ecosystems (Stoecker *et al.* 2009). Second, many heterotrophic protists living in oligotrophic waters have established obligatory symbioses with photosynthetic microalgae, generating a mixotrophic consortium (Decelle *et al.* 2015; Not *et al.* 2016). Finally, the majority of eukaryotic microalgae have absorbotrophic abilities, but their capacity for uptake of organic carbon vs. non-carbon elements remains poorly quantified (Flynn *et al.* 2013).

Necrotrophic mixotrophy (phagotrophy) in aquatic ecosystems

Microalgae can feed on other organisms to supplement their needs in carbon and non-carbon elements by predation through

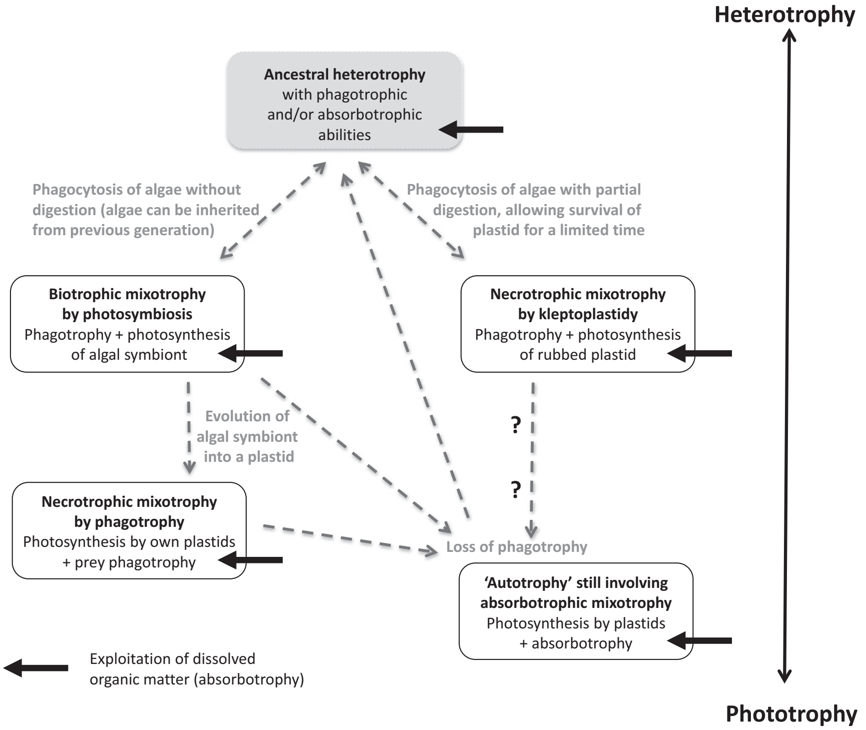

Figure 4 Diversity of the mixotrophic strategies in aquatic algae (white boxes), with evolutionary pathways (dotted arrows) indicating evolution from heterotrophic ancestors (grey box at the top). The arrow indicates the widespread absorbotrophic nutrition on dissolved organic matter.

phagocytosis (Fig. 4). Increasing evidence and analytic methods (Box 3) suggest that this is the rule, rather than the exception in aquatic environments. In necrotrophic mixotrophy, the predatory relationship between the organisms involved is straightforward, and most aquatic research scientists consider it as core mixotrophy. Although uncommon among land plants, predation is widespread in microalgae (Raven *et al.* 2009; Flynn *et al.* 2013): phagotrophy has long been recognised in taxa such as chrysophytes and prymnesiophytes and may also be prevalent in cryptophytes and dinoflagellates (Stoecker 1998; Fig. 1). Field and laboratory experiments have revealed many examples of bacterial grazing by microalgae (e.g. Jeong *et al.* 2010; Wilken *et al.* 2013), but phagotrophic microalgae may also feed on other prey, such as other microalgae or even metazoans. *Karlodinium armiger*, a mixotrophic dinoflagellate, attacks copepods, bivalve larvae and finfish (Berge *et al.* 2012), by collective swarming behaviour (chemotaxis) and paralysis of the prey with neurotoxins, followed by sucking out by a feeding tube. Predation by green microalgae is assumed to be rare but occurs at least in Pyramimonadales (Bell & Laybourn-Parry 2003; Maruyama & Kim 2013; McKie-Krisberg & Sanders 2014). Even the coccolithophorid prymnesiophytes *Emiliania huxleyi*, an intensively studied and key microalgal taxon in temperate oceans, long considered purely photosynthetic, can capture bacteria using a characteristic filamentous apparatus called haptonema (Rokitta *et al.* 2011). All previously cited taxa share a motile apparatus implying at least one flagella-like structure and have traditionally been lumped together in 'phytoflagellates' or 'autotrophic flagellates' by aquatic microbiologists. Only diatoms so far lack species with necrotrophic mixotrophy, probably because their extracellular silica cell wall, which allows exchange only through small pores, prevents phagocytosis.

Necrotrophic mixotrophy was for a long time linked to oligotrophic conditions where non-carbon elements are limiting and considered relevant at the individual level only. Necrotrophic mixotrophy turns out to play key roles in global ecology (Unrein *et al.* 2007; Zubkov & Tarran 2008; Moorthi *et al.* 2009; Hartmann *et al.* 2012; Ward & Follows 2016). Phagotrophic microalgae can account for more than half of the pigmented biomass and are responsible for a large part, sometime the largest, of the bacterivory by flagellated cells in planktonic ecosystems (Havskum & Riemann 1996). Necrotrophic mixotrophy has now been well documented for nutrient-rich environments too, where lower light levels can stimulate the grazing abilities of microalgae mainly for carbon uptake (Hansen & Hjorth 2002; Burkholder *et al.* 2008). Until recently, quantitative studies focused largely on specific species known to perform necrotrophic mixotrophy and to have an impact on the environment, e.g., harmful algal blooms species such as *Prymnesium*, dinoflagellates (Jeong *et al.* 2004; Stoecker *et al.* 2006; Graneli *et al.* 2012), or focused on the estimated grazing of the total pigmented flagellate populations (Hitchman & Jones 2000; Unrein *et al.* 2007). Few studies have been able to sort out the relative contributions of distinct taxonomic groups to global necrotrophic mixotrophy in the environment, although small haptophytes consistently turn out to be significant bacterial grazers (Unrein *et al.* 2014), a feature that may explain their widespread occurrence in the oceans (Liu *et al.* 2009).

A similar situation is found in species that use plastids stolen from free-living algae. In this strategy called

Box 3 Tools to study phagotrophy and absorbotrophy in aquatic environments

Phagotrophy requires active uptake of particulate matter (aggregates or cells), its digestion and metabolic use by the consumer. The most widely used technique to study the *in situ* uptake of organic particles by phagotrophic protists employs isotopic or fluorescent-labelled particles as food tracers in short-term incubations. Labelling of bacteria with ^{35}S-methionine or ^{3}H-leucine enables estimates of bacterivory rates from radioactivity recovered in protist cells (Zubkov & Tarran 2008). By coupling radiolabelling of the natural bacterioplankton and flow cytometry sorting of phagotrophic cells, Hartmann *et al.* (2012) demonstrated that plastidic protists, rather than the ones without plastids (i.e. heterotrophic), control bacterivory in the surface mixed layer of the Atlantic Ocean. Techniques for the fluorescent labelling of food tracers, such as fluorescent microspheres (Børsheim 1984) or fluorescently labelled bacteria (FLB), have also been used (Sherr *et al.* 1987). The accumulation of labelled particles in the food vacuole of a grazer provides an uptake rate for food ingestion. The major advantages of the FLB technique are the use of natural bacteria and the possibility to count labelled bacteria in food vacuoles from a single host cell under the microscope, as well as the possibility of rough identification of grazers based on morphology. Because of the difficulty of simultaneous estimation of the *in situ* grazing rates and identification of these tiny organisms, very few studies have attempted to discriminate phagotrophic cells by taxonomic affiliation (Riemann *et al.* 1995; Havskum & Riemann 1996). Recent studies have identified and quantified mixotrophic taxa smaller than 20 μm thanks to specific DNA probes by fluorescent *in situ* hybridisation (Hartmann *et al.* 2013; Unrein *et al.* 2014). The combination of short-term grazing experiments using FLB and FISH identification was used to estimate the grazing rate of various microalgae. Most studies, however, lack evidence for trophic utilisation of ingested particles. Frias-Lopez *et al.* (2009) used an RNA stable isotope probing technique (RNA-SIP). Protists were fed over short periods with cyanobacteria pre-cultivated in^{13}C- and ^{15}N-labelled seawater samples and then submitted to RNA extraction. Separation of 'heavy' (labelled) from 'light' (unlabelled) RNA by density gradient ultracentrifugation allowed recovery of DNA from eukaryotic cells that consumed the labelled cyanobacteria. Then, 18S rRNA amplification allowed phylogenetic affiliation of mixotrophs, but unfortunately RNA-SIP does not permit quantification of bacterivory rates.

Considering *absorbotrophy*, some microalgal species have for decades been known to utilise dissolved organic compounds (Neilson & Lewin 1974). Most studies, however, used axenic batch cultures in the dark or light with rather high initial concentrations of organic substrate. Direct measurements under natural conditions are confronted by two major issues: first, the complex composition and low concentration of dissolved organic matter in the environment, and second, the complex communities of natural samples that contain varying proportions of potential users of organic compounds: zooplankton, microalgae, heterotrophic protists and bacteria. Size fractionation of *in situ* samples (Berman 1975), cell sorting by micromanipulation (Rivkin & Putt 1987) and the use of inhibitors of prokaryotic metabolism (Iturriaga & Zsolnay 1981) enable partial reduction in the biological complexity. The chemical complexity remains unsolved because the substrates used by different algae, even when closely related, differ considerably (Neilson & Lewin 1974), and this prevents prediction of substrates that can be used by a given taxon of microalgae. The technique most widely used to study absorbotrophy of photosynthetic cells employs isotopic-tagged organic molecules. The subsequent determination of heavy or radioactive isotopes recovered in cells allows estimation of *in situ* uptake rates of any given substrates. The serial extraction of different algal molecules (Rivkin & Voytek 1987) even enables study of the labelling patterns of polymers among different microalgal species, different environmental conditions (light/dark) and different labelled organic substrates (Rivkin & Putt 1987).

kleptoplastidy, an algal prey is ingested and partially digested, providing carbon and non-carbon elements, with the exception of its plastids. The latter remain undigested and active in predator's host cells, where they supplement the carbon need. Kleptoplastidy evolved in many unrelated unicellular groups such as Foraminifera, Ciliates and in animals such as Acoel worms and molluscs (Johnson *et al.* 2007; Stoecker *et al.* 2009; Cruz *et al.* 2013). Plastid survival is limited in time and in contrast to endosymbiosis kleptoplastidy can be considered somehow as a delayed predation: we thus consider this evolutionary dead end as a necrotrophy. Kleptoplastid longevity within the host differs from one model to another, ranging from a few days to several months (up to 1 year in the foraminifer *Nonionella stella,* Grzymski *et al.* 2002). Although horizontal gene transfers from the algal to the host nucleus were suggested to support the activity of plastids by allowing the renewal of key proteins (Rumpho *et al.* 2008), transcriptomic studies have led to contradictory results (e.g. Wägele *et al.* 2011) suggesting alternative hypotheses, including intrinsic plastid stability (Pillet 2013; Raven 2015). Keptoplastidic organisms probably use a combination of different mechanisms to maintain the plastids of their algal prey and clearly improve the carbon to non-carbon ratio gained from prey.

Biotrophic mixotrophy (photosymbiosis) in aquatic ecosystems

Biotrophic mixotrophy in aquatic organisms fundamentally differs from land plants. In contrast to terrestrial ecosystems where the autotroph is larger and considered as the host and the heterotroph as the symbiont (e.g. in mycorrhizae), an opposite situation prevails in aquatic ecosystems, where the autotrophic microalgae are the symbionts, often included as endosymbionts within a heterotrophic protist considered as the host (Stoecker *et al.* 2009; Decelle *et al.* 2015; Fig. 4). Such biotrophic mixotrophy is also called photosymbiosis because it makes photosynthesis indirectly accessible to the host. Photosynthetic endosymbionts can be inherited from the previous generation or repetitively acquired from the environment by phagocytosis, although mixed scenarios also exist (Okamoto & Inouye 2006). Among the hosts, heterotrophic protists from several lineages such as Foraminifera, Ciliates and Radiolaria encompass many representatives bearing microalgal endosymbionts (Fig. 1), but photosymbiosis also involves some large animals gaining photosynthetic abilities through intracellular algal symbionts (Venn *et al.* 2008; Bailly *et al.* 2014). Iconic examples of such symbioses are the associations between corals or jellyfishes with diverse endosymbiotic microalgae, usually dinoflagellates, commonly named zooxanthellae. These animals can harbour millions of algal cells per cm^3 of tissue that are obligatory for the host's survival through food supply, but which also enhance calcification in reef-building corals, thereby acting as architect species for the whole ecosystem (Dubinsky & Stambler 2011). Less perceptible to inexperienced people, yet among the first ever symbiotic relationships described back in 1881 by Karl Brandt (Brandt 1881; Probert *et al.* 2014), photosymbioses involving two protists are widespread and can be extremely significant for ecosystem functioning (Stoecker *et al.* 2009; Decelle *et al.* 2015; Not *et al.* 2016): indeed, biotrophic mixotrophy, and in particular photosymbioses, are common in oligotrophic environments, where they are assumed to constitute a short cut in the food web (Taylor 1982; Norris 1996; Biard *et al.* 2016). In such associations, the heterotrophic host acquires carbon through its algal endosymbiont photosynthates and the symbiont takes advantage of significant quantities of non-carbon nutrients produced by the digestive processes of the host. Studies performed on Radiolaria have conservatively estimated that 9–16% of the carbon incorporated by the symbionts during 4 h is translocated to the host (Anderson *et al.* 1983; Box 3). However, essentially because of the lack of appropriate tools, very few studies have investigated quantitatively the carbon and non-carbon fluxes between partners in biotrophic mixotrophy of planktonic organisms and the exact contribution of photosynthesis to the host's carbon budget. Photosymbioses can have a significant ecological impact through their important contribution to biomass and primary production (Michaels 1988; Stoecker *et al.* 1996, 2009; Dennett *et al.* 2002; Biard *et al.* 2016). In the equatorial Pacific, photosymbiotic hosts contribute 27, 47 and 56% of the total allegedly heterotrophic cell biomass in the 20–64, 64–200 and > 200 μm size classes respectively (Stoecker *et al.* 1996). The total production of microalgae from photosymbioses typically contributes *ca.* 1% of the total primary production in surface waters, but occasionally accounts for up to 20% during periods of high host cell densities (Michaels 1988; Caron *et al.* 1995). Biotrophic mixotrophy is, however, a particularly risky trophic behaviour, as most of the time there is no vertical transmission of the symbionts, which implies *de novo* acquisition from the environment at each host generation.

Absorbotrophic mixotrophy in aquatic ecosystems

As for land plants, the vast majority of microalgal groups investigated so far exhibit absorbotrophic uptake of organic matter, of natural or anthropogenic sources (Fig. 4). There is evidence that this supplements their requirement in non-carbon elements, although experimental approaches (e.g. by isotopic labelling) sometime do not investigate loss of dissolved organic compounds from photosynthetic microalgae, and thus do not assess net influx. This includes uptake of amino acids and other compounds such as urea for nitrogen supply to a variety of algal species (Burkholder *et al.* 2008). Some microalgae with specific vitamin requirements may take them up directly from dissolved substances (Croft *et al.* 2006). Substrates can be acquired by various means such as direct uptake or following extracellular enzymatic degradation (e.g. peptidases) and hydrolysis (Stoecker & Gustafson 2003). Urea has been particularly studied as it is considered as an indicator of coastal runoff and anthropogenic release of organic matter and non-carbon nutrients through intensive animal farming and cropland fertiliser application. The relationship between high urea concentration and increased harmful algal bloom occurrences has been demonstrated (e.g. Glibert & Legrand 2006). In estuarine ecosystems, urea contributes nearly 45% of the total nitrogen uptake by microalgal assemblages (Twomey *et al.* 2005).

Although mixotrophy is usually associated with oligotrophic environmental settings, absorbotrophic mixotrophy is commonly found in meso- to eutrophic waters where organic matter abounds and thus this trophic mode would be one of the explanations for massive harmful algal bloom occurrences in some ecosystems (Burkholder *et al.* 2008). The direct uptake of high-molecular-weight macromolecules, likely through pinocytosis, has been demonstrated for a couple of dinoflagellates, but its contribution to microalgal nutrition remains unclear (Granéli *et al.* 1999). As for land plants, absorbotrophic mixotrophy is essentially considered as a strategy to acquire mainly organic non-carbon elements, and competing bacteria quickly assimilate most low-molecular-weight organic carbon. Estimations for carbon acquisition by this means are currently lacking. Algae can indeed use various carbon substrates, including acetate and related compounds (lactate or pyruvate), ethanol, fatty acids, glycolate, glycerol and sugars, but use less diverse sources than bacteria (Neilson & Lewin 1974), and are often unable to grow as pure heterotrophs in the dark.

Absorbotrophic mixotrophy makes sense for primarily autotrophic organisms as, by definition, phagotrophs have to acquire their nutrients through feeding by ingestion. Yet a number of heterotrophic protists, primarily grazers of prokaryotes or microalgae, also perform absorbotrophy (Sanders 1992; Fig. 4), so that absorbotrophy is likely an ancestral trait. For instance, absorbotrophic uptake of radiolabelled dimethylsulfoniopropionate, a common organosulfur compound produced by microalgae, has been demonstrated experimentally for the heterotrophic dinoflagellate *Oxyrrhis marina* (Saló *et al.* 2009). Although this remains to be quantified more precisely, absorbotrophic mixotrophy may be extremely important for microalgal ecology and more generally for the functioning of aquatic ecosystems.

Summary

Aquatic mixotrophy encompasses a large diversity of eukaryotic lineages (Fig. 1). While it arose by multiple derived traits on land, it is mostly based on the ancestral phagocytic feature in aquatic ecosystems (Fig. 4), which allows phagotrophic predation or establishment of photosymbioses, although rare innovations exist (such as the peduncle in some mixotrophic dinoflagellates; Yoo *et al.* 2010). Indeed, phagotrophy is easily achievable in water, but more difficult in terrestrial ecosystems where cells are more often protected by walls. Another difference between terrestrial and aquatic mixotrophy is that terrestrial mixotrophs also borrow water during mixotrophy, although this is a non-limiting factor for aquatic mixotrophs.

Despite accumulating evidence, the significance of mixotrophy in aquatic ecosystem functioning is underestimated and poorly quantified. Its key role has still not fully percolated throughout the aquatic sciences and is not properly appreciated by ecologists, despite increasing calls for attention (Flynn *et al.* 2013; Worden *et al.* 2015; Mitra *et al.* 2016). Yet, the ecological relevance is emerging faster for aquatic mixotrophy than for terrestrial mixotrophy, for which we lack consideration and ecological data.

MIXOTROPHY IS EVERYWHERE

Ubiquity and plasticity

The various modes of mixotrophy (Box 1), whether on land or in aquatic environments, are widespread from phylogenetic (Fig. 1) and ecological perspectives (Figs 3 and 4). Their physiological meaning encompasses two non-exclusive *raisons d'être*: obtaining carbon and non-carbon elements such as nitrogen, phosphorus or iron through organic matter (Fig. 3). At the ecophysiological level, there is evidence that mixotrophy is plastic and allows adaptation to the available level of carbon or mineral nutrients. The rate of heterotrophic use of organic resources depends on light or nutrient availability in protists (Flöder *et al.* 2006; Smalley *et al.* 2012; Unrein *et al.* 2014), and it depends on light level in some terrestrial mixotrophs (Preiss *et al.* 2010; Matsuda *et al.* 2012; Gonneau *et al.* 2015; Box 2). Its relevance for accommodating to the level of oligotrophy is established for carnivorous and some hemiparasitic plants. As a result, individual mixotrophic species acclimate to a range of light vs. non-carbon element balances (Katechakis *et al.* 2005). The mixotrophic abilities of many species are thus much more complex than the simple addition of autotrophy and heterotrophy: they can be finely tuned and constantly shift along a continuum of nutritional strategies that can be balanced according to the physiological requirements over a large range of environmental availabilities of light and non-carbon elements. Such shifts make it particularly difficult to conceptualise energy flow within a mixotrophic organism and consequently to incorporate quantitative information into a functional model of the food web (e.g. for plankton; Flynn & Mitra 2009; Mitra & Flynn 2010; Stoecker 1998). Aquatic mixotrophy creates major issues for ecosystem modelling by biasing energy flow in classic food web structures, distinguishing auto- and heterotrophs only (Tittel *et al.* 2003; Mitra & Flynn 2010; Mitra *et al.* 2016; Ward & Follows 2016).

Mixotrophy as an evolutionary bridge between auto- and heterotrophy

Mixotrophy is intermediate between auto- and heterotrophy in evolutionary terms. It allows a reversion to heterotrophy in autotrophs, as shown by the loss of photosynthesis in lineages issuing from hemiparasitic and partially mycoheterotrophic mixotrophic land plants. Multiple reversions to heterotrophy through mixotrophy also occurred in algal lineages (Stoecker 1998; Figueroa-Martinez *et al.* 2015). Experimental evolution shows that *Chlamydomonas* (a green microalga that can use acetate to fulfil 50% of its carbon needs; Heifetz *et al.* 2000; Tittel *et al.* 2005) evolves a pure absorbotrophic heterotrophy when cultured in the dark and provided with organic substrate, not only increasing its adaptation to heterotrophic growth but sometimes even reducing or losing its ability to grow in the light (Bell & Adams 2011). Mixotrophy is also a way to autotrophy, especially when heterotrophic protists acquire and maintain a functional plastid or an entire photosynthetic organism (Fig. 4). Indeed, biotrophic mixotrophy probably paved the way to the multiple origins of plastids in the evolution of eukaryotes (Fig. 1). The ongoing acquisition of new plastids in diverse lineages of heterotrophic dinoflagellates illustrates this process (Gagat *et al.* 2013). This is why mixotrophy based on phagotrophy in planktonic algae can be seen as a persistence of the ancestral phagotrophic mechanism that allowed plastid acquisition (Maruyama & Kim 2013; Fig. 4).

Mixotrophy may, however, entail additional costs to support the two trophic modes. Raven (1997) evaluated that mixotrophic protists require five times more energy and nutrient allocation to maintain the photosynthetic apparatus as compared with maintaining a strictly heterotrophic feeding apparatus. Whenever organic matter is constantly available, the costs of maintaining simultaneously photosynthetic capacities may outweigh the benefits, and then mixotrophy is at risk of progressively evolving into heterotrophy, as shown by experimental evolution on *Chlamydomonas* (Bell 2012). However, heterotrophic abilities can also be costly, e.g., capturing devices required for biotrophic and necrotrophic mixotrophy can be more physiologically demanding. For example, traps of carnivorous plants are costly (Adamec 2006) and reduce investment in photosynthetic tissues (Ellison & Gotelli 2009). In phagocytic mixotrophs, the cell wall cannot be as continuous and rigid as in fully autotrophic cells, and thus is less protective. Finally, hemiparasitic mixotrophs in productive environments exemplify the conditional cost of mixotrophy, as they are poorly competitive for light under such conditions where other plants grow profusely (Těšitel *et al.* 2011). In cases where both heterotrophy and autotrophy are costly, mixotrophy is at risk of progressively evolving into pure heterotrophy or pure autotrophy, whenever, respectively, organic matter or light are permanently available. In some cases, absorbotrophic mixotrophy can be achieved at low physiological cost, because it is a by-product of other functions: for instance, whenever multicellular land plants or large algae have some non-photosynthetic cells, absorbotrophic abilities have already been acquired as a way for these cells to take up nutrients from the internal organismal environment (Schaffelke 1999). Yet mixotrophic strategies are diverse, and thus raise the question of what ecological conditions may affect the balance of costs and benefice towards a persistence of mixotrophy. We explore below under what conditions mixotrophy is advantageous.

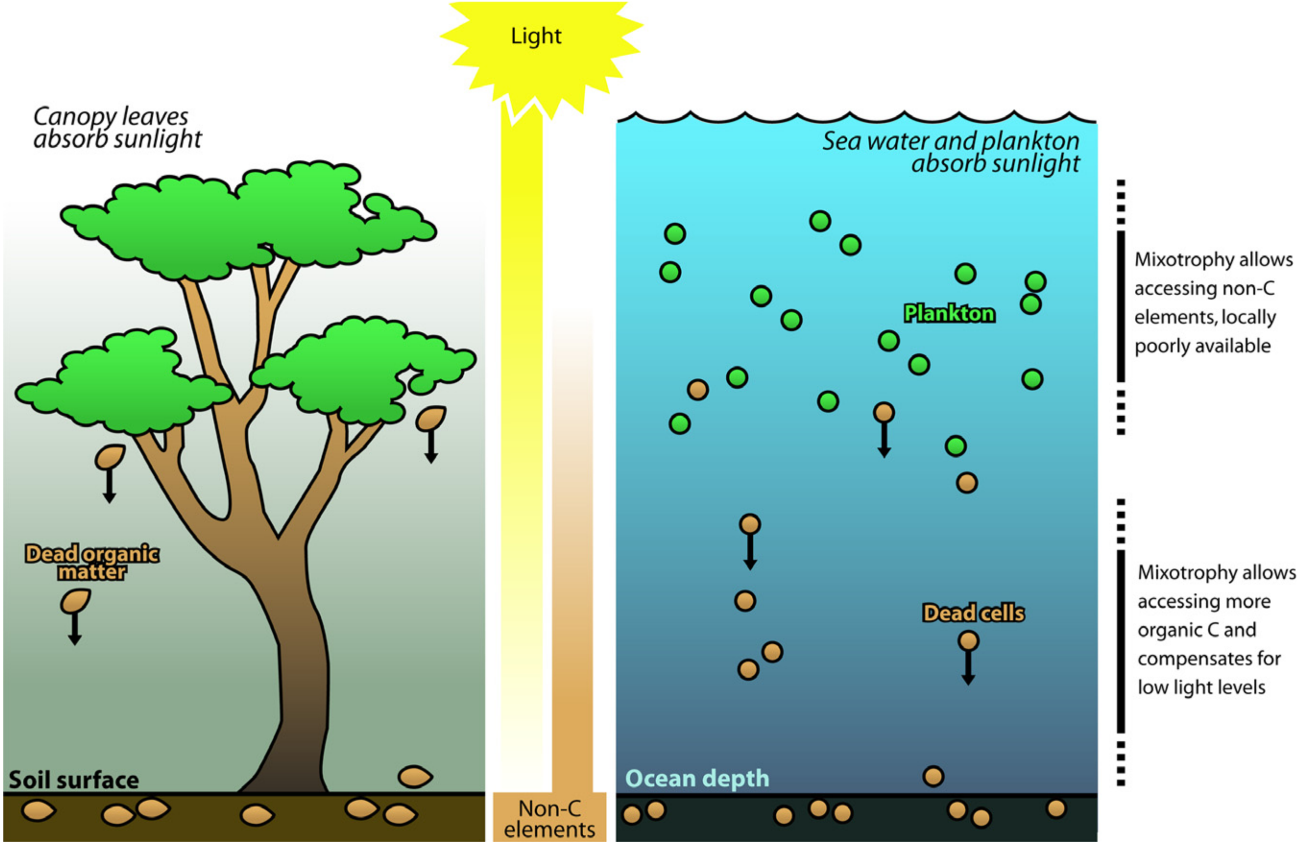

Figure 5 The *grand écart* hypothesis as a driver for emergence and persistence of mixotrophy. The *grand écart* is a general framework of imbalance between light and non-carbon elements that favours growth of mixotrophs and selects for them. Biological mechanisms create inverse gradients of light and mineral resources in all ecosystems, terrestrial (due to the formation of a canopy, left) and aquatic (due to the biological pump and water column stratification, right): as a result, non-carbon elements tend to accumulate at the bottom of the ecosystem, under organic and/or inorganic forms. Mixotrophy may adapt to the various conditions of the resulting ecosystem layers (design, courtesy by Aurélien Miralles).

The persistence and diversity of mixotrophy implies that selective advantages compensate for extra physiological costs. Some mechanisms have been proposed to maintain mixotrophy in specific lineages. For example, intrinsic constraints in terrestrial mixotrophs limit the shift to heterotrophy. Isotopic methods (Box 2) suggest that carbon heterotrophically obtained from plants by hemiparasites or from mycorrhizal fungi by partial mycoheterotrophs is used for vegetative growth, while photosynthesis contributes to produce seeds (Pageau *et al.* 1998; Santos-Izquierdo *et al.* 2008; Roy *et al.* 2013; Bellino *et al.* 2014). Thus, mixotrophy is meta-stable because fitness is reduced by loss of photosynthesis and the emergence of full heterotrophy is possible only after complex, and rarely achieved, physiological modifications. A second example is given by some planktonic mixotrophs that compete for the same prey with their own predators. This competition reduces food availability to below the minimal threshold required for a pure heterotroph, and gives mixotrophs an advantage (Tittel *et al.* 2003). Finally, as stated above, nutrient-poor environments globally favour mixotrophy, which gives access to non-carbon elements; it has been proposed that, in the polar regions, the absence of light selects for mixotrophy (Kirkham *et al.* 2013; McKie-Krisberg & Sanders 2014). Yet, environment-, niche- or lineage-restricted reasoning fails to account for the widespread distribution of mixotrophy.

The '*grand écart*' hypothesis

We suggest that a general property of ecosystem dynamics selects for the ubiquitous emergence and persistence of mixotrophy. In terrestrial ecosystems, plants exploit compartmentalised resources: light and gas, including CO_2, in the atmosphere vs. non-carbon elements in soil, obtained either directly or through mycorrhizae. While in pioneer stages a reduced biomass allows organisms to live close to the interface between air and soil, biomass accumulation with ecological successions progressively entails increasing competition for light. In the resulting shrubby or forest ecosystems, erect trunks are required to access the light, now situated away from the soil resources (Fig. 5, left panel). A gradient is thus established, from a canopy layer (with light but devoid of non-carbon elements) to a ground layer (nearby non-carbon elements but shaded).

In aquatic ecosystems, the upper layers of the water column receive light (the 'photic' layer). In continental and coastal waters, non-carbon elements are provided by terrestrial inputs, but in open oceans, i.e., most of the Earth's surface, non-carbon elements (nitrogen, phosphorus and mainly iron) are sparse due to limited input. Two additional factors enhance the segregation of non-carbon elements far from light. A first, abiotic factor is the stratification of the water column during the summer, with warm and light water at the surface, not mixing with deeper, colder and denser layers. This thermal stratification limits the recycling of sinking particles and nutrients throughout the water column. The second factor is a biologically driven depletion of upper water layers as dead organic cells sink to deeper layers and export both carbon and non-carbon elements. The so-called biological pump enhances depletion of non-carbon elements in the photic zone. A gradient is thus established (Fig. 5, right panel) similar to the terrestrial one from a photic layer with light but devoid of non-carbon elements to deeper layers richer in non-carbon resources but shaded. Even if, in some regions at specific time of the year, the layers of the water column experience mixing events destabilising such gradients, planktonic cells are nonetheless often selected for mixotrophy when conditions become stratified and the *grand écart* is established.

Thus, the biological dynamics of most ecosystems split light and non-carbon resources, by successions that establish shrubs and trees in terrestrial ecosystems or by biological pump in aquatic ecosystems. Organisms therefore face a '*grand écart*' between non-carbon and light resources (Fig. 5). The French word *grand écart* (the name for a dancer's splits) is commonly used to express the need to combine two poorly compatible tasks to maintain a difficult position. We argue that ecosystem structures achieved by ecological successions on land and the biological pump and water column stratification in aquatic ecosystems provide a range of situations where mixotrophy can be selected (Fig. 5), in spite of its costs. The *grand écart* is a general framework of imbalance between light and non-carbon elements that favours growth of mixotrophs, and selects from them. Mixotrophy is selected for higher carbon gains in shaded layers, e.g., forest understory mixotrophs using carbon from mycorrhizal fungi or planktonic mixotrophs in deep water layers supplementing their low photosynthesis by partial heterotrophy. At the other extreme, mixotrophy is selected for access to non-carbon elements from biotic sources in nutrient-poor layers, e.g., carnivorous and symbiotic or hemiparasitic plants living epiphytically in the forest canopy or microalgae performing phagotrophy at the oligotrophic surface of aquatic ecosystems (lakes and oceans). The balance between light and non-carbon elements of course changes in time and space, occasionally favouring specialist autotrophs and heterotrophs, but often favours mixotrophs.

Mixotrophy in turn amplifies the *grand écart* by fuelling the gradients. In terrestrial ecosystems, mixotrophic epiphytes enhance forest canopy density (at least in tropical forests), while understored mixotrophic plants derive non-carbon elements away from the canopy. In aquatic ecosystems, large mixotrophic cells sink more rapidly after death than their smaller prey would have, thus accelerating the biological pump (Richardson & Jackson 2007; Mitra *et al.* 2014; Ward & Follows 2016). Finally, ecosystem dynamics likely played a direct role in the evolution from autotrophy to mixotrophy. In aquatic environments, seasonal stratification cyclically pushes autotrophs into conditions increasingly favourable to mixotrophy (reduction in availability of non-carbon elements). Similarly, during successions in terrestrial ecosystems, herbaceous autotrophs that initially develop before the increase of competition for light are pushed into conditions favouring heterotrophy (absence of light but high organic carbon abundance). Thus, the repeated establishment of the *grand écart* conditions enhances the evolutionary transition from autotrophy to mixotrophy.

CONCLUSION AND FUTURE RESEARCH

The classic opposition between heterotrophy and autotrophy turns out to be an inappropriate framework for most photosynthetic organisms and a number of phagotrophs. Most of them should be placed in a continuum ranging from full autotrophy to full heterotrophy (e.g. Fig. 4), at a position that can vary quantitatively according to environmental conditions. The *grand écart* accounts for the widespread occurrence of mixotrophy in today's ecosystems. It also accounts for the shifts from auto- to heterotrophy, and vice versa, observed in eukaryotic evolution and which are allowed through mixotrophic evolutionary intermediates.

Until very recently, the classic view opposing heterotrophy to autotrophy has limited the evaluation of the physiological and ecological relevance of mixotrophy, and this remains particularly true in terrestrial ecosystems. We now need more quantification of mixotrophic processes (e.g. elemental fluxes and budgets) in order to be able to understand better the implications of mixotrophy for ecology on a global scale. Mixotrophy is clearly crucial for aquatic ecosystem functioning. For instance, mixotrophic behaviour across a light gradient in the photic layer of aquatic systems would structure the biotic communities and, along with abiotic parameters, would be responsible for the establishment of the deep chlorophyll maximum (Tittel *et al.* 2003). Investigations on ecological relevance are pending for terrestrial mixotrophs and have so far only been considered for hemiparasites (Press & Phoenix 2005; Watson 2009; Bell & Adams 2011). A particular question is how mixotrophy will be impacted and respond under rapidly changing environmental conditions. Recent studies estimate that climate change is perturbing this unstable equilibrium and will have a negative impact on mixotrophy by leaning towards either strict autotrophy or strict heterotrophy (Caron & Hutchins 2013; Wilken *et al.* 2013).

In terms of evolution, the investigation of mixotrophic vs. fully autotrophic or heterotrophic abilities in fine-scale phylogenies will evaluate the evolutionary role of mixotrophy as an intermediate towards auto- or heterotrophy, as well as its meta-stability (Figueroa-Martinez *et al.* 2015). For instance, some experiments demonstrate the role of mixotrophy in the resilience of a photosynthetic biosphere under catastrophically darkened conditions across geological time (Jones *et al.* 2009). Considering mixotrophy at a broader level, aquatic models are used to address its impact on carbon and nutrient cycles (e.g. Ward & Follows 2016), while terrestrial models question more the impact of mixotrophy on community structure. In the future, both scientific communities may interact more and draw inspiration from this discrepancy in order to address ecological questions, and engage in joined-up quantitative experiments and modelling about mixotrophy everywhere.

ACKNOWLEDGEMENTS

We thank Lubomir Adamec, Aaron Ellison, Kevin Flynn, Jakub Těšitel, John Raven and Colomban de Vargas for discussions, three anonymous referees for helpful suggestions, Aurélien Miralles for re-designing Fig. 5 for us and David Marsh for English corrections. This research has been supported by the 2015/18/A/NZ8/00149 grant funded by National Science Centre (Poland).

AUTHORSHIP

M.-A.S. proposed the idea of the *grand écart* hypothesis and all authors equally contributed to write the paper (M.-A.S. mainly as terrestrial ecologist; M.C. and F.N. mainly as aquatic ecologists) and prepared the figures and boxes.

GLOSSARY

Absorbotrophy: (also called osmotrophy, although osmotic forces are not the sole drivers) the uptake of low-molecular-weight substances by way of passive or active transport at the cell membrane level.

Anaplerotic reactions: metabolic reactions that form intermediates of a metabolic pathway, e.g., the biosynthesis of a carboxylic acid by way of carboxylation of a substrate with CO_2.

Biotrophic: interspecific trophic interaction, mutualistic or parasitic, where the two partners stay alive.

Carnivory: the eating and/or digesting of animal biomass.

Endosymbiosis: a symbiosis in which the cell of one partner is embedded in that of the other. Such symbiosis gives rise to plastids.

Grand écart: (the name for a dancer's splits) is a metaphor for situations where it is difficult to combine two optimal traits that are antagonistic – such as access to light vs. non-carbon elements in many ecosystems.

Hemiparasite: parasitic plant that is photosynthetic but exploits the xylem sap from other plants, where it collects non-carbon and also carbon elements.

Kleptoplastidy: a strategy where full or partial autotrophy is acquired by way of sequestering plastids from other organisms; a kind of delayed predation since such plastids have limited survival.

Microalgae: Photosynthetic unicellular organisms, microscopic algae, e.g., abundant in the plankton.

Mixotrophy: a nutrition that combines heterotrophy and autotrophy, i.e., obtains organic matter by way of photosynthesis and uptake of pre-existing organic matter in the environment, as a source of carbon or non-carbon elements.

Mycoheterotrophy: a heterotrophic nutrition of terrestrial plants that relies on carbon provided by fungi colonising the roots and forming mycorrhizae (see this word).

Mycorrhiza: a dual symbiotic, often mutualistic, organ formed by a plant root and a soil fungus. In 90% of land plants, mycorrhizae allow the exchange of soil water and mineral nutrients, from the fungus, for sugars from the plant. In some plants, a modified mechanism allows the plant also to collect carbon from the fungus (mycoheterotrophy), which questions the mutualism in such cases.

Necrotrophy: interspecific trophic interaction, mutualistic or parasitic, which entails the death of one of the partners during nutrition.

Phagotrophy: the uptake of food particles by engulfment in the cell by phagocytosis (or endocytosis). While the fusion of lysosomes often follows and digests the particles, the resulting intracellular vesicles can also stay intact for a limited period (e.g. in kleptoplastidy) or permanently over cellular generations (e.g. in endosymbiosis).

Photosymbiosis: A symbiosis that unites a heterotrophic and an autotrophic organism, usually an endosymbiosis where a unicellular autotroph is engulfed in a heterotrophic host cell. In eukaryotic evolution, this scenario gave rise to plastids.

Symbiosis: an interspecific interaction, mutualistic or parasitic, where the two partners live together.

REFERENCES

Abadie, J.-C., Püttsepp, Ü., Gebauer, G., Faccio, A., Bonfante, P. & Selosse, M.-A. (2006). *Cephalanthera longifolia* (Neottieae, Orchidaceae) is mixotrophic: a comparative study between green and nonphotosynthetic individuals. *Can. J. Bot*., 84, 1462–1477.

Adamec, L. (1997). Mineral nutrition of carnivorous plants: a review. *Bot. Rev*., 63, 273–299.

Adamec, L. (2006). Respiration and photosynthesis of bladders and leaves of aquatic *Utricularia* species. *Plant Biol*., 8, 765–769.

Adlassnig, W., Koller-Peroutka, M., Bauer, S., Koshkin, E., Lendl, T. & Lichtscheidl, I.K. (2012). Endocytotic uptake of nutrients in carnivorous plants. *Plant J*., 71, 303–313.

Anderson, B. (2005). Adaptations to foliar absorption of faeces: a pathway in plant carnivory. *Annals Bot*., 95, 757–761.

Anderson, O.R., Swanberg, N.R. & Bennett, P. (1983). Assimilation of symbiont-derived photosynthates in some solitary and colonial Radiolaria. *Mar. Biol*., 77, 265–269.

Ashley, T. & Gennaro, J.F. (1971). Fly in the sundew. *Nat. Hist. (New York)*, 80, 80–85.

Bailly, X., Laguerre, L., Correc, G., Dupont, S., Kurth, T., Pfannkuchen, A. *et al*. (2014). The chimerical and multifaceted marine acoel *Symsagittifera roscoffensis*: from photosymbiosis to brain regeneration. *Front. Microb*., 5, 498.

Baldauf, S.L. (2008). An overview of the phylogeny and diversity of eukaryotes. *J. Syst. Evol*., 46, 263–273.

Barrett, C.F., Freudenstein, J.V., Li, J., Mayfield-Jones, D.R., Perez, L., Pires, J.C. *et al*. (2014). Investigating the path of plastid genome degradation in an early-transitional clade of heterotrophic orchids, and implications for heterotrophic angiosperms. *Mol. Biol. Evol*., 31, 3095–3112.

Behie, S.W., Zelisko, P.M. & Bidochka, M.J. (2012). Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. *Science*, 336, 1576–1577.

Bell, G. (2012). Experimental evolution of heterotrophy in a green alga. *Evolution*, 67, 468–476.

Bell, T. & Adams, M. (2011). Attack on all fronts: functional relationships between aerial and root parasitic plants and their woody hosts and consequences for ecosystems. *Tree Physiol*., 31, 3–15.

Bell, E.M. & Laybourn-Parry, J. (2003). Mixotrophy in the Antarctic phytoflagellate, *Pyramimonas gelidicola* (Chlorophyta: Prasinophyceae). *J. Phycol*., 39, 644–649.

Bellino, A., Baldantoni, D., Selosse, M.-A., Guerrieri, R., Borghetti, M. & Alfani, A. (2014). Photosynthesis regulation in mixotrophic plants: new insights from *Limodorum abortivum*. *Oecologia*, 175, 875–885.

Berge, T., Poulsen, L.K., Moldrup, M., Daugbjerg, N. & Juel Hansen, P. (2012). Marine microalgae attack and feed on metazoans. *ISME J*., 6, 1926–1936.

Berman, T. (1975). Size fractionation of natural aquatic populations associated with autotrophic and heterotrophic carbon uptake. *Mar. Biol.*, 33, 215–220.

Biard, T., Stemmann, L., Picheral, M., Mayot, N., Vandromme, P., Hauss, H. *et al.* (2016). *In situ* imaging reveals the biomass of giant protists in the global ocean. *Nature*, 532, 504–507.

Bidartondo, M.I., Burghardt, B., Gebauer, G., Bruns, T.D. & Read, D.J. (2004). Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *Proc. Roy. Soc. B*, 271, 1799–1806.

Bolin, J.F., Tennakoon, K.U., Majid, M.B.A. & Cameron, D.D. (2016). Isotopic evidence of partial mycoheterotrophy in *Burmannia coelestis* (Burmanniaceae). *Plant Sp. Biol.*, in press.

Borowicz, V.A. & Armstrong, J.E. (2012). Resource limitation and the role of a hemiparasite on a restored prairie. *Oecologia*, 169, 783–792.

Børsheim, K.Y. (1984). Clearance rates of bacteria-sized particles by freshwater ciliates, measured with monodisperse fluorescent latex beads. *Oecologia*, 63, 286–288.

Brandt, K. (1881). Uber das Zusammenleben von Thieren und Algen. *Verh. Physiol. Ges.*, 1881–2, 22–26.

Braukmann, T., Kuzmina, M. & Stefanovic, S. (2013). Plastid genome evolution across the genus *Cuscuta* (Convolvulaceae): two clades within subgenus *Grammica* exhibit extensive gene loss. *J. Exp. Bot.*, 64, 977–989.

Bréda, N., Maillard, P., Montpied, P., Bréchet, C., Garbaye, J. & Courty, P.E. (2013). Isotopic evidence in adult oak trees of a mixotrophic lifestyle during spring reactivation. *Soil Biol. Biochem.*, 58, 136–139.

Burkholder, J.M., Glibert, P.M. & Skelton, H.M. (2008). Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae*, 8, 77–93.

Caron, D.A. & Hutchins, D.A. (2013). The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *J. Plank. Res.*, 35, 235–252.

Caron, D.A., Michaels, A.F., Swanberg, N.R. & Howse, F.A. (1995). Primary productivity by symbiont-bearing planktonic Sarcodines (Acantharia, Radiolaria, Foraminifera) in surface waters near Bermuda. *J. Plank. Res.*, 17, 103–129.

Cernusak, L.A., Tcherkez, G., Keitel, C., Cornwell, W.K., Santiago, L.S., Knohl, A. *et al.* (2009). Why are non-photosynthetic tissues generally ^{13}C enriched compared with leaves in C3 plants? Review and synthesis of current hypotheses. *Funct. Pl. Biol.*, 36, 199–213.

Chase, M.W., Christenhusz, M.J.M., Sanders, D. & Fay, M.F. (2009). Murderous plants: Victorian Gothic, Darwin and modern insights into vegetable carnivory. *Bot. J. Linn. Soc.*, 161, 329–356.

Courty, P.E., Walder, F., Boller, T., Ineichen, K., Wiemken, A. & Selosse, M.-A. (2011). C and N metabolism in mycorrhizal networks and mycoheterotrophic plants of tropical forests: a stable isotope analysis. *Plant Physiol.*, 156, 952–961.

Courty, P.E., Doubková, P., Calabrese, S., Niemann, H., Lehmann, M.F., Vosátka, M. & Selosse, M.-A. (2015). Species-dependent partitioning of C and N stable isotopes between arbuscular mycorrhizal fungi and their C3 and C4 hosts. *Soil Biol. Bioch.*, 82, 52–61.

Croft, M.T., Warren, M.J. & Smith, A.G. (2006). Algae need their vitamins. *Eukaryot. Cell*, 5, 1175–1183.

Cruz, S., Calado, R., Serôdio, J. & Cartaxana, P. (2013). Crawling leaves: photosynthesis in sacoglossan sea slugs. *J. Exp. Bot.*, 64, 3999–4009.

Darwin, C. (1875). *Insectivorous Plants*. John Murray, London, pp. 1–462.

Dearnaley, J.D.W., Martos, F. & Selosse, M.-A. (2012). Orchid mycorrhizas: Molecular ecology, physiology, evolution and conservation aspects. In *Fungal associations, The Mycota IX*. (ed Hock, B.). Springer, Berlin, Germany, pp. 207–230.

Decelle, J., Colin, S. & Foster, R.A. (2015). Photosymbiosis in marine planktonic protists. In *Marine Protists: Diversity and Dynamics* (eds Ohtsuka, O., Suzaki, T., Horiguchi, T., Suzuki, N., Not, F.). Springer, Japan, pp. 465–500.

Defossez, E., Selosse, M.-A., Dubois, M.-P., Mondolot, L., Faccio, A., Djieto-Lordon, C. *et al.* (2009). Ant-plants and fungi: a new threeway symbiosis. *New Phytol.*, 182, 942–949.

Defossez, E., Djieto-Lordon, C., McKey, D., Selosse, M.A. & Blatrix, R. (2011). Plant-ants feed their host plant, but above all a fungal symbiont to recycle nitrogen. *Proc. Roy. Soc. B*, 278, 1419–1426.

Dennett, M.R., Caron, D.A., Michaels, A.F., Gallager, S.M. & Davis, G.S. (2002). Video plankton recorder reveals high abundances of colonial Radiolarians in surface waters of the central North Pacific. *J. Plank. Res.*, 24, 797–805.

Dixon, K.W., Pate, J.S. & Bailey, W.J. (1980). Nitrogen nutrition of the tuberous sundew *Drosera erythrorhiza* Lindl. with species reference to catch of arthropod fauna by its glandular leaves. *Aust. J. Bot.*, 28, 283–297.

Dubinsky, Z. & Stambler, N. (2011). *Coral Reefs: An Ecosystem in Transition*. Springer, Netherlands, pp. 1–521.

Ellison, A.M. & Gotelli, N.J. (2002). Nitrogen availability alters the expression of carnivory in the northern pitcher plant *Sarracenia purpurea*. *Proc. Nat Acad. Sci. USA*, 99, 4409–4412.

Ellison, A.M. & Gotelli, N.J. (2009). Energetics and the evolution of carnivorous plants—Darwin's 'most wonderful plants in the world'. *J. Exp. Bot.*, 60, 19–42.

Eriksson, O. & Kainulainen, K. (2011). The evolutionary ecology of dust seeds. *Persp. Plant Ecol. Evol. Syst.*, 13, 73–87.

Farquhar, G.D., Ehleringer, J.R. & Hubrck, K.T. (1989). Carbon isotope discrimination and photosynthesis. *Ann. Rev. Pl. Phys. Pl. Mol. Biol.*, 40, 503–537.

Field, K.J., Leake, J.R., Tille, S., Allinson, K.E., Rimington, W.R., Bidartondo, M.I. *et al.* (2015). From mycoheterotrophy to mutualism: mycorrhizal specificity and functioning in *Ophioglossum vulgatum* sporophytes. *New Phytol.*, 205, 1492–1502.

Figueroa-Martinez, F., Nedelcu, A.M., Smith, D.R. & Reyes-Prieto, A. (2015). When the lights go out: the evolutionary fate of free-living colorless green algae. *New Phytol.*, 206, 972–982.

Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R.A., Henrissat, B. *et al.* (2012). The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science*, 336, 1715–1719.

Flynn, K.J. & Mitra, A. (2009). Building the "perfect beast": modelling mixotrophic plankton. *J. Plankt. Res.*, 31, 965–992.

Flynn, K.J., Stoecker, D.K., Mitra, A., Raven, J.A., Glibert, P.M., Hansen, P.J. *et al.* (2013). Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *J. Plank. Res.*, 35, 3–11.

Frias-Lopez, J., Thompson, A., Waldbauer, J. & Chisholm, S.W. (2009). Use of stable isotope-labelled cells to identify active grazers of picocyanobacteria in ocean surface waters. *Env. Microbiol.*, 11, 512–525.

Flöder, S., Hansen, T. & Ptacnik, R. (2006). Energy–dependent bacterivory in Ochromonas minima – A strategypromoting the use of substitutable resources and survival at insufficient light supply. *Protist*, 157, 291–302.

Gagat, P., Bodył, A., Mackiewicz, P. & Stiller, J.W. (2013). Tertiary plastid endosymbioses in Dinoflagellates. In *Endosymbiosis*. (ed Löffelhardt, W.). Springer, Berlin, Heidelberg, pp. 233–290.

Gebauer, G. & Meyer, M. (2003). N-15 and C-13 natural abundance of autotrophic and mycoheterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytol.*, 160, 209–223.

Girlanda, M., Selosse, M.-A., Cafasso, D., Brilli, F., Delfine, S., Fabbian, R. *et al.* (2006). Inefficient photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. *Mol. Ecol.*, 15, 491–504.

Glibert, P.M. & Legrand, C. (2006). The diverse nutrient strategies of harmful algae: focus on osmotrophy. In *Ecology of Harmful Algae, Ecological Studies* (eds Granéli, P.D.E., Turner, P.D.J.T.). Springer, Berlin, Heidelberg, pp. 163–175.

Gonneau, C., Jersáková, J., de Tredern, E., Till-Bottraud, I., Saarinen, K., Sauve, M. *et al.* (2015). Photosynthesis in perennial mixotrophic

Epipactis spp. (Orchidaceae) contributes more to shoot and fruit biomass than to hypogeous survival. *J. Ecol.*, 102, 1183–1194.

Granéli, E., Carlsson, P. & Legrand, C. (1999). The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. *Aq. Ecol.*, 33, 17–27.

Granéli, E., Edvardsen, B., Roelke, D.L. & Hagström, J.A. (2012). The ecophysiology and bloom dynamics of *Prymnesium* spp. *Harmful Algae*, 14, 260–270.

Grzymski, J., Schofield, O.M. & Falkowski, P.G. (2002). The function of plastids in the deep-sea benthic foraminifer, Nonionella stella. *Limnol. Oceanogr.*, 47, 1569–1580.

Hansen, P.J. & Hjorth, M. (2002). Growth and grazing responses of *Chrysochromulina ericina*. *Mar. Biol.*, 141, 975–983.

Hartmann, M., Grob, C., Tarran, G.A., Martin, A.P., Burkill, P.H., Scanlan, D.J. *et al.* (2012). Mixotrophic basis of Atlantic oligotrophic ecosystems. *Proc. Nat Acad. Sci. USA*, 109, 5756–5760.

Hartmann, M., Zubkov, M.V., Scanlan, D.J. & Lepère, C. (2013). *In situ* interactions between photosynthetic picoeukaryotes and bacterioplankton in the Atlantic Ocean: evidence for mixotrophy. *Environ. Microb. Rep.*, 5, 835–840.

Hashimoto, Y., Fukukawa, S., Kunishi, A., Suga, H., Richard, F. *et al.* (2012). Mycoheterotrophic germination of *Pyrola asarifolia* dust seeds reveals convergences with germination in orchids. *New Phytol.*, 195, 620–630.

Havskum, H. & Riemann, B. (1996). Ecological importance of bacterivorous, pigmented flagellates (mixotrophs) in the Bay of Aarhus, Denmark. *Mar. Ecol. Prog. Ser.*, 137, 251–263.

Heifetz, P.B., Förster, B., Osmond, C.B., Giles, L.J. & Boynton, J.E. (2000). Effects of acetate on facultative autotrophy in *Chlamydomonas reinhardtii* assessed by photosynthetic measurements and stable isotope analyses. *Pl. Physiol.*, 122, 1439–1445.

van der Heijden, M.G.A., Martin, F., Selosse, M.-A. & Sanders, I.R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol.*, 205, 1406–1423.

Hitchman, R.B. & Jones, H.L.J. (2000). The role of mixotrophic protists in the population dynamics of the microbial food web in a small artificial pond. *Freshwater Biol.*, 43, 231–241.

Hynson, N.A., Madsen, T.P., Selosse, M.-A., Adam, I.K.U., Ogura-Tsujita, Y., Roy, M. *et al.* (2013). The physiological ecology of mycoheterotrophy. In *Mycoheterotrophy*. (ed Merckx, V.S.F.T.). Springer, New York, pp. 297–342.

Iturriaga, R. & Zsolnay, A. (1981). Differentiation between auto- and heterotrophic activity: problems in the use of size fractionation and antibiotics. *Bot. Mar.*, 24, 399–404.

Jeong, H.J., Yoo, Y.D., Kim, J.S., Kim, T.H., Kim, J.H., Kang, N.S. *et al.* (2004). Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophycean): prey species, the effects of prey concentration, and grazing impact. *J. Euk. Microbiol.*, 51, 563–569.

Jeong, H.J., Yoo, Y.D., Kim, J.S., Seong, K.A., Kang, N.S. & Kim, T.H. (2010). Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Sci. J.*, 45, 65–91.

Johnson, M.D., Oldach, D., Delwiche, C.F. & Stoecker, D.K. (2007). Retention of transcriptionally active cryptophyte nuclei by the ciliate *Myrionecta rubra*. *Nature*, 445, 426–428.

Jones, H., Cockle, C.S., Goodson, C., Price, N., Simpson, A. & Thomas, B. (2009). Experiments on mixotrophic protists and catastrophic darkness. *Astrobiology*, 9, 563–571.

Julou, T., Burghardt, B., Gebauer, Y., Berveiller, D., Damesin, C. & Selosse, M.-A. (2005). Mixotrophy in orchids: insights from a comparative study of green individuals and non-photosynthetic individuals of *Cephalanthera damasonium*. *New Phytol.*, 166, 639–653.

Karagatzides, J.D., Butler, J.L. & Ellison, A.M. (2009). The pitcher plant *Sarracenia purpurea* can directly acquire organic nitrogen and short-circuit the inorganic nitrogen cycle. *PLoS ONE*, 4, e6164.

Katechakis, A., Haseneder, T., Kling, R. & Stibor, H. (2005). Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: the light: nutrient hypothesis might not hold for mixotrophs. *Limnol. Oceanogr.*, 50, 1290–1299.

Kirkham, A.R., Lepère, C., Jardillier, L.E., Not, F., Bouman, H., Mead, A. *et al.* (2013). A global perspective on marine photosynthetic picoeukaryote community structure. *ISME J.*, 7, 922–936.

Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F. *et al.* (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat. Genet.*, 47, 410–415.

Kuyper, T.W. & Kiers, E.T. (2014). The danger of mycorrhizal traps? *New Phytol.*, 203, 352–354.

Lallemand, F., Gaudeul, M., Lambourdière, J., Matsuda, Y., Hashimoto, Y. & Selosse, M.-A. (2016). The elusive predisposition to mycoheterotrophy in Ericaceae. *New Phytol.*, 212, 314–319.

Liu, H., Probert, I., Uitz, J., Claustre, H., Aris-Brosou, S., Frada, M. *et al.* (2009). Extreme diversity in non-calcifying haptophytes explains a major pigment paradox in open oceans. *Proc. Nat Acad. Sci. USA*, 106, 12803–12808.

Maruyama, S. & Kim, E. (2013). A modern descendant of early green algal phagotrophs. *Cur. Biol.*, 23, 1081–1084.

Matsuda, Y., Shimizu, S., Mori, M., Ito, S.I. & Selosse, M.-A. (2012). Seasonal and environmental changes of mycorrhizal associations and heterotrophy levels in mixotrophic *Pyrola japonica* (Ericaceae) growing under different light environments. *Am. J. Bot.*, 99, 1177–1188.

McKie-Krisberg, Z.M. & Sanders, R.W. (2014). Phagotrophy by the picoeukaryotic green alga Micromonas: implications for Arctic Oceans. *ISME J.*, 8, 1953–1961.

McNeal, J.R., Bennett, J.R., Wolfe, A.D. & Mathews, S. (2013). Phylogeny and origins of holoparasitism in Orobanchaceae. *Am. J. Bot.*, 100, 971–983.

Merckx, V., Stöckel, M., Fleischmann, A., Bruns, T.D. & Gebauer, G. (2010). ^{15}N and ^{13}C natural abundance of two mycoheterotrophic and a putative partially mycoheterotrophic species associated with arbuscular mycorrhizal fungi. *New Phytol.*, 188, 590–596.

Michaels, A.F. (1988). Vertical distribution and abundance of Acantharia and their symbionts. *Mar. Biol.*, 97, 559–569.

Mitra, A. & Flynn, K.J. (2010). Modelling mixotrophy in harmful algal blooms: more or less the sum of the parts? *J. Mar. Syst.*, 83, 158–169.

Mitra, A., Flynn, K.J., Burkholder, J.M., Berge, T., Calbet, A., Raven, J.A. *et al.* (2014). The role of mixotrophic protists in the biological carbon pump. *Biogeosciences*, 11, 995–1005.

Mitra, A., Flynn, K.J., Tillmann, U., Raven, J.A., Caron, D., Stoecker, D.K. *et al.* (2016). Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition: incorporation of diverse mixotrophic strategies. *Protist*, 167, 106–120.

Moorthi, S., Caron, D.A., Gast, R.J. & Sanders, R.W. (2009). Mixotrophy: a widespread and important ecological strategy for planktonic and sea-ice nanoflagellates in the Ross Sea. *Antarctica*. *Aq. Microb. Ecol.*, 54, 269–277.

Näsholm, T., Kielland, K. & Ganeteg, U. (2009). Uptake of organic nitrogen by plants. *New Phytol.*, 182, 31–48.

Neilson, A.H. & Lewin, R.A. (1974). The uptake and utilization of organic carbon by algae: an essay in comparative biochemistry. *Phycologia*, 13, 227–264.

Nishi, A.H., Vasconcellos-Neto, J. & Romero, G.Q. (2013). The role of multiple partners in a digestive mutualism with a protocarnivorous plant. *Ann. Bot.*, 111, 143–150.

Norris, R.D. (1996). Symbiosis as an evolutionary innovation in the radiation of Paleocene planktic foraminifera. *Paleobiol.*, 22, 461–480.

Not, F., Siano, R., Kooistra, W.H.C.F., Simon, N., Vaulot, D. & Probert, I. (2012). Diversity and ecology of eukaryotic marine phytoplankton. In: *Advances in Botanical Research*. (ed. Piganeau, G.). Elsevier, Amsterdam, Netherlands, pp. 1–53.

Not, F., Probert, I., Ribeiro, C.G., Crenn, K., Guillou, L., Jeanthon, C. *et al.* (2016). Photosymbiosis in marine pelagic environments. In *The*

Marine Microbiome (eds Stal, L.J., Cretoiu, M.S.). Springer International Publishing, Switzerland, pp. 305–332.

Okamoto, N. & Inouye, I. (2006). *Hatena arenicola* gen. et sp. nov., a katablepharid undergoing probable plastid acquisition. *Protist*, 157, 401–419.

Orwin, K.H., Kirschbaum, M.U.F., St John, M.G. & Dickie, I.A. (2011). Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecol. Lett*., 14, 493–502.

Pageau, K., Simier, P., Naulet, N., Robins, R. & Fer, A. (1998). Carbon dependency of the hemiparasite *Striga hermonthica* on *Sorghum bicolor* determined by carbon isotopic and gas exchange analyses. *Aust. J. Pl. Physiol*., 25, 695–700.

Paungfoo-Lonhienne, C., Lonhienne, T.G.A., Rentsch, D., Robinson, N., Christie, M., Webb, R.I. *et al*. (2008). Plants can use protein as a nitrogen source without assistance from other organisms. *Proc. Nat Acad. Sci. USA*, 105, 4524–4529.

Paungfoo-Lonhienne, C., Rentsch, D., Robatzek, S., Webb, R., Sagulenko, E., Näsholm, T. *et al*. (2010). Turning the table: plants consume microbes as a source of nutrients. *PLoS ONE*, 5, e11915.

Pillet, L. (2013). The role of horizontal gene transfer in kleptoplastidy and the establishment of photosynthesis in the eukaryotes. *Mob. Gen. Elements*, 3, e24773.

Preiss, K., Adam, I.K.U. & Gebauer, G. (2010). Irradiance governs exploitation of fungi, fine-tuning of carbon gain by two partially mycoheterotrophic orchids. *Proc. Roy. Soc. B*, 277, 1333–1336.

Press, M.C. & Phoenix, G.K. (2005). Impacts of parasitic plants on natural communities. *New Phytol*., 166, 737–751.

Press, M., Smith, S. & Stewart, G. (1991). Carbon acquisition and assimilation in parasitic plants. *Funct. Ecol*., 5, 278–283.

Probert, I., Siano, R., Poirier, C., Decelle, J., Biard, T., Tuji, A. *et al*. (2014). *Brandtodinium* gen. nov. and *B. nutricula* comb. nov. (Dinophyceae), a dinoflagellate commonly found in symbiosis with polycystine radiolarians. *J. Phycol*., 50, 388–399.

Quested, H.M. (2008). Parasitic plants—impacts on nutrient cycling. *Plant Soil*, 311, 269–272.

Raven, J.A. (1997). Phagotrophy in phototrophs. *Limnol. Oceanogr*., 42, 198–205.

Raven, J.A. (2015). Implications of mutation of organelle genomes for organelle function and evolution. *J. Exp. Bot*., 66, 5639–5650.

Raven, J.A., Beardall, J., Flynn, K.J. & Maberly, S.C. (2009). Phagotrophy in the origins of photosynthesis in eukaryotes and as a complementary mode of nutrition in phototrophs: relation to Darwin's insectivorous plants. *J. Exp. Bot*., 60, 3975–3987.

Read, D.J. & Kerley, S.J. (1995). The status and function of ericoid mycorrhizal systems. In *Mycorrhiza* (eds Hock, B., Varma, A.). Springer-Verlag, Heidelberg, pp. 499–520.

Richardson, T.L. & Jackson, G.A. (2007). Small phytoplankton and carbon export from the surface ocean. *Science*, 315, 838–840.

Riemann, B., Havskum, H., Thingstad, F. & Bernard, C. (1995). The role of mixotrophy in pelagic environments. In: *Molecular Ecology of Aquatic Microbes* (eds Joint, I.). The NATO ASI Series, Springer. Berlin, Heidelberg, pp. 87–114.

Rineau, F., Shah, F., Smits, M.M., Persson, P., Johansson, T., Carleer, R. *et al*. (2013). Carbon availability triggers the decomposition of plant litter and assimilation of nitrogen by an ectomycorrhizal fungus. *ISME J*., 7, 2010–2022.

Rischer, H., Hammv, A. & Brinkmann, G. (2002). *Nepenthes insignis* Danser (Nepenthaceae) uses a C2-portion of the carbon skeleton of alanine acquired via its carnivorous organs to build up the allelochemical plumbagin. *Phytochemistry*, 59, 603–609.

Rivkin, R.B. & Putt, M. (1987). Heterotrophy and photoheterotrophy by Antarctic microalgae: light dependant incorporation of amino acids and glucose. *J. Phycol*., 23, 442–452.

Rivkin, R.B. & Voytek, M.A. (1987). Photoadaptations of photosynthesis and carbon metabolism by phytoplankton from McMurdo Sound, Antarctica. 1. Species-specific and community responses to reduced irradiances. *Limnol. Oceanogr*., 32, 249–259.

Rokitta, S.D., de Nooijer, L.J., Trimborn, S., de Vargas, C., Rost, B. & John, U. (2011). Transcriptome analyses reveal differential gene expression patterns between the life-cycle stages of *Emiliania huxleyi* (Haptophyta) and reflect specialization to different ecological niches. *J. Phycol*., 47, 829–838.

Rousk, J., Hill, P.W. & Jones, D.L. (2014). Using the concentration-dependence of respiration arising from glucose addition to estimate *in situ* concentrations of labile carbon in grassland soil. *Soil Biol. Biochem*., 77, 81–88.

Roy, M., Gonneau, C., Rocheteau, A., Berveiller, D., Thomas, J.-C., Damesin, C. *et al*. (2013). Why do mixotrophic plants stay green? A comparison between green orchid individuals *in situ*. *Ecol. Monog*., 83, 95–117.

Rumpho, M.E., Worful, J.M., Lee, J., Kannan, K., Tyler, M.S., Bhattacharya, D. *et al*. (2008). Horizontal gene transfer of the algal nuclear gene psbO to the photosynthetic sea slug *Elysia chlorotica*. *Proc. Nat Acad. Sci. USA*, 105, 17867–17871.

Saló, V., Simó, R., Vila-Costa, M. & Calbet, A. (2009). Sulfur assimilation by *Oxyrrhis marina* feeding on a ^{35}S-DMSP-labelled prey. *Environ. Microb*., 11, 3063–3072.

Sanders, R.W. (1992). Trophic strategies among heterotrophic flagellates. In: *The Biology of Free-Living Heterotrophic Flagellates* (eds Patterson, D.J. & Larsen, J.), Clarendon Press, Oxford, pp. 21–38.

Santos-Izquierdo, B., Pageau, K., Fer, A., Simier, P. & Robins, R.J. (2008). Targeted distribution of photo-assimilate in *Striga hermonthica* (Del.) Benth parasitic on *Sorghum bicolor* L. *Phytochem. Lett*., 1, 76–80.

Schaffelke, B. (1999). Particulate organic matter as an alternative nutrient source for tropical Sargassum species (Fucales, Phaeophyceae). *J. Phycol*., 35, 1150–1157.

Schmidt, S.A., Raven, J.A. & Paungfoo-Lonhienne, C. (2013). The mixotrophic nature of photosynthetic plants. *Funct. Plant Biol*., 40, 425–438.

Selosse, M.-A. & Martos, F. (2014). Do chlorophyllous orchids heterotrophically use mycorrhizal fungal carbon?. *Trends Pl. Sci*., 19, 683–685.

Selosse, M.-A. & Roy, M. (2009). Green plants that feed on fungi: facts and questions about mixotrophy. *Trends Pl. Sci*., 14, 64–70.

Selosse, M.-A., Richard, F., He, X. & Simard, S. (2006). Mycorrhizal networks: les liaisons dangereuses. *Trends Ecol. Evol*., 21, 621–628.

Selosse, M.-A., Bocayuva, M.F., Kasuya, M.C.M. & Courty, P.-E. (2016). Mixotrophy in mycorrhizal plants: extracting C from mycorrhizal networks. In *Molecular Mycorrhizal Symbiosis*. (ed Martin, F.). Springer, Berlin, Germany, pp. 451–471.

Sherr, B.F., Sherr, E.B. & Fallon, R.D. (1987). Use of monodispersed fluorescently labeled bacteria to estimate *in situ* protozoan bacterivory. *Appl. Env. Microbiol*., 53, 958–965.

Simard, S.W., Beiler, K.J., Bingham, M.A., Deslippe, J.R., Philip, L.J. & Teste, F.P. (2012). Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biol. Rev*., 26, 39–60.

Smalley, G.W., Coats, D.W. & Stoecker, D.K. (2012). Influence of inorganic nutrients, irradiance, and time of day on food uptake by the mixotrophic dinoflagellate *Neoceratium furca*. *Aquat. Microb. Ecol*., 68, 29–41.

Stoecker, D.K. (1998). Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Eur. J. Protistol*., 34, 281–290.

Stoecker, D.K. & Gustafson, D.E. (2003). Cell-surface proteolytic activity of photosynthetic dinoflagellates. *Aquat. Microb. Ecol*., 30, 175–183.

Stoecker, D.K., Gustafson, D.E. & Verity, P.G. (1996). Micro- and mesoprotozooplankton at 140°W in the equatorial Pacific: heterotrophs and mixotrophs. *Aquat. Microb. Ecol*., 10, 273–282.

Stoecker, D., Tillmann, U. & Granéli, E. (2006). Phagotrophy in harmful algae. In *Ecology of Harmful Algae, Ecological Studies* (eds Granéli, P.D.E., Turner, P.D.J.T.). Springer, Berlin, Heidelberg, pp. 177–187.

Stoecker, D., Johnson, M., deVargas, C. & Not, F. (2009). Acquired phototrophy in aquatic protists. *Aquat. Microb. Ecol*., 57, 279–310.

Taylor, F.J.R. (1982). Symbioses in Marine Microplankton. *Ann. Inst. Océanogr.*, 58, 61–90.

Tedersoo, L., Pellet, P., Kõljalg, U. & Selosse, M.-A. (2007). Parallel evolutionary paths to mycoheterotrophy in understorey Ericaceae and Orchidaceae: ecological evidence for mixotrophy in Pyroleae. *Oecologia*, 151, 206–217.

Těšitel, J. (2016). Functional biology of parasitic plants: a review. *Pl. Ecol. Evol.*, 149, 5–20.

Těšitel, J., Plavcová, L. & Cameron, D.D. (2010). Interactions between hemiparasitic plants and their hosts: the importance of organic carbon transfer. *Plant Signal. Behav.*, 5, 1072–1076.

Těšitel, J., Lepš, J., Vráblová, M. & Cameron, D.D. (2011). The role of heterotrophic carbon acquisition by the hemiparasitic plant *Rhinanthus alectorolophus* in seedling establishment in natural communities: a physiological perspective. *New Phytol.*, 192, 188–199.

Tittel, J., Bissinger, V., Zippel, B., Gaedke, U., Bell, E., Lorke, A. *et al.* (2003). Mixotrophs combine resource use to outcompete specialists: implications for aquatic food webs. *Proc. Nat Acad. Sci. USA*, 100, 12776–12781.

Tittel, J., Bissinger, V., Gaedke, U. & Kamjunke, N. (2005). Inorganic carbon limitation and mixotrophic growth in *Chlamydomonas* from an acidic mining lake. *Protist*, 156, 63–75.

Treseder, K.K., Davidson, D.W. & Ehleringer, J.R. (1995). Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature*, 375, 137–139.

Treseder, K.K., Torn, M.S. & Masiello, C.A. (2006). An ecosystem-scale radiocarbon tracer to test use of litter carbon by ectomycorrhizal fungi. *Soil Biol. Biochem.*, 38, 1077–1082.

Trudell, S.A., Rygiewicz, P.T. & Edmonds, R.L. (2003). Nitrogen and carbon stable isotope abundances support the myco-heterotrophic nature and host-specificity of certain achlorophyllous plants. *New Phytol.*, 160, 391–401.

Twomey, L.J., Piehler, M.F. & Paerl, H.W. (2005). Phytoplankton uptake of ammonium, nitrate and urea in the Neuse River Estuary, NC, USA. *Hydrobiol.*, 533, 123–134.

Unrein, F., Massana, R., Alonso-Sáez, L. & Gasol, J.M. (2007). Significant year-round effect of small mixotrophic flagellates on bacterioplankton in an oligotrophic coastal system. *Limnol. Oceanogr.*, 52, 456–469.

Unrein, F., Gasol, J.M., Not, F., Forn, I. & Massana, R. (2014). Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. *ISME J.*, 8, 164–176.

Venn, A.A., Loram, J.E. & Douglas, A.E. (2008). Photosynthetic symbioses in animals. *J. Exp. Bot.*, 59, 1069–1080.

Wägele, H., Deusch, O., Händeler, K., Martin, R., Schmitt, V., Christa, G. *et al.* (2011). Transcriptomic evidence that longevity of acquired plastids in the photosynthetic slugs *Elysia timida* and *Plakobranchus ocellatus* does not entail lateral transfer of algal nuclear genes. *Mol. Biol. Evol.*, 28, 699–706.

Wang, L., Kgope, B., D'Odorico, B. & Macko, S.A. (2008). Carbon and nitrogen parasitism by xylem-tapping mistletoe (*Tapinanthus oleifolius*) along the Kalahari Transect: a stable isotope study. *Afr. J. Ecol.*, 46, 540–546.

Ward, B.A. & Follows, M.J. (2016). Marine mixotrophy increases trophic transfer efficiency, mean organism size, and vertical carbon flux. *Proc. Nat Acad. Sci. USA*, 113, 2958–2963.

Watson, D.M. (2009). Parasitic plants as facilitators: more Dryad than Dracula? *J. Ecol.*, 97, 1151–1159.

Westwood, J.H., Yoder, J.I., Timko, M.P. & dePamphilis, C.W. (2010). The evolution of parasitism in plants. *Trends Plant Sci.*, 15, 227–235.

Wilken, S., Huisman, J., Naus-Wiezer, S. & Van Donk, E. (2013). Mixotrophic organisms become more heterotrophic with rising temperature. *Ecol. Lett.*, 16, 225–233.

Worden, A.Z., Follows, M.J., Giovannoni, S.J., Wilken, S., Zimmerman, A.E. & Keeling, P.J. (2015). Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. *Science*, 347, 1257594.

Yoo, Y.D., Jeong, H.J., Kang, N.S., Song, J.Y., Kim, K.Y., Lee, G. *et al.* (2010). Feeding by the newly described mixotrophic dinoflagellate *Paragymnodinium shiwhaense*: feeding mechanism, prey species, and effect of prey concentration. *J. Euk. Microbiol.*, 57, 145–158.

Zubkov, M.V. & Tarran, G.A. (2008). High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. *Nature*, 455, 224–226.

Editor, Punidan Jeyasingh
Manuscript received 11 July 2016
First decision made 22 August 2016
Second decision made 27 October 2016
Manuscript accepted 13 November 2016