Impact of climate warming on carbon metabolism and on morphology of invasive and native aquatic plant species varies between spring and summer

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Impact of climate warming on carbon metabolism and on morphology of invasive and native aquatic plant species varies between spring and summer

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Highlights

- ● A 3°C increase was applied on two invasive and two native species at two seasons
- ● The warming induced changes in morphological traits and in carbohydrate contents
- ● The growth of the invasive species was particularly stimulated by the warming in spring
- ● Carbohydrate patterns suggest common physiological mechanisms for the two invasives
- ● Climate warming may favor the colonisation by invasive species over native species

Abstract

The rise of global surface temperature by between 1.2°C and 4°C by 2100 is expected to affect freshwater ecosystems and the growth of aquatic plants. By extending the distribution range of invasive macrophytes, climate warming could increase their management costs. The aim of this study was to test the impact of a 3°C warming in spring and in summer on the morphology and physiology

of two native species (*Mentha aquatica*, *Myosotis scorpioides*) and two invasive species (*Ludwigia hexapetala*, *Myriophyllum aquaticum*) under controlled conditions. Our study showed that the increase of spring temperature induced morphological modifications for all species, while a 3°C warming induced changes in carbohydrates composition for native species in spring, and modification of carbohydrate content for invasive species at both seasons. Patterns of carbohydrate content group the two invasive species together, possibly highlighting common physiological mechanisms. Moreover, the increase of spring temperature favoured the apical and/or lateral growth solely for invasive species. Hence, the invasive species specific response to warming suggests that higher temperature may favour their growth in spring, which might allow them to colonise the water column earlier than natives. This competitive advantage could affect aquatic ecosystems functioning and biodiversity in the coming years.

Keywords: biological invasion; climate change; functional traits; macrophytes; metabolic profiling; photoassimilates

1. Introduction

Biological invasions and climate warming are two of the major ecological concerns of the 21st century. In its 5th report, the IPCC estimates that temperatures will increase by between 1.2°C and 4°C by 2100 (Collins et al., 2013). Impacts of warmer temperatures on ecosystems will be varied (IPCC, 2014) and could promote future biological invasions (Bellard et al., 2013). Invasive species are exotic species that spread widely when growing in their introduced ranges. Their high colonisation capacities can be explained, among other reasons, by their high plasticity to environmental conditions (Walther et al., 2009), by their successful competition abilities (Vilà and Weiner, 2004) or by the release from enemy pressure (Keane and Crawley, 2002). Thus, their geographical range would be expected to increase with increased temperature due to climate change (Bellard et al., 2013; Clements and Ditommaso, 2011). Although challenging, the study of the responses of invasive

species to climate warming will improve our understanding of their short- or long-term effects on ecosystems. This will help to predict their adaptive strategies.

Many experimental studies have investigated the impact of warmer climates on invasive terrestrial plants (Carón et al., 2015; Verlinden et al., 2014). Aquatic ecosystems are also sensitive to biological invasions and to climate change (Dudgeon et al., 2006; Rahel and Olden, 2008; Sala et al., 2000), but are less studied. Most aquatic invasive plant species form dense mats on the water surface (Hussner, 2009). This intrudes on human activities in aquatic environments, by hampering fishing and swimming in ponds or boat traffic on rivers (Hussner and Lösch, 2007; Zhang and Boyle, 2010). As temperature influences the growth of all plants and productivity of some macrophyte species (Barko et al., 1982; Madsen and Brix, 1997), studying the responses of invasive macrophytes to warmer temperatures is necessary to understand how future climate could increase the invasion risk. Moreover, native species may also benefit from future temperature warming, and improve both their growth and spatial distribution. For example (McKee et al., 2002) showed that for the native *Potamogeton natans,* flowering occurred earlier in the season and the leaf surface area increased under warming. Nonetheless, as invasive species can invade climate envelopes quite different than those from their area of origin (Gallagher et al., 2010), the introduced populations may differ in their ability to adjust to changing climate. Their plasticity and their local adaptation can confer them a competitive advantage over native species during adaptation to climate warming.

Several authors have highlighted the importance of physiological ecology for a better understanding of climate change impacts on organisms (Bozinovic and Pörtner, 2015; Brunetti et al., 2013; Denny and Helmuth, 2009). Indeed, functional traits are widely used to characterise plant response to climate change (Nicotra et al., 2010), but physiological responses of plants to climate warming are more rarely considered, but mainly by studying the impact of elevated temperature (≈40°C) on crop species (DaMatta et al., 2010). Metabolic profiling analysis represents an opportunity to study physiological responses of organisms by detailed characterisation of plant metabolic responses to

various environmental perturbations (Lisec et al., 2006). For example, Pagter *et al.* (2011) highlighted qualitative and quantitative changes in carbohydrates for *Hydrangea* species in response to temperature. This approach has also been used to study changes induced by abiotic factors on invasive freshwater plant metabolism, revealing a reorientation of the primary metabolism in response to salt stress, especially for carbohydrates (Thouvenot et al., 2015). Thus, metabolic profiling provides a more precise knowledge about the effects of abiotic stress on plants, complementary with functional traits. Furthermore, such an approach generates complex datasets whose the processing and the synthesis constitute a challenge. In the field of biological invasions, metabolomic studies can help to determine potential physiological mechanisms underlying invasion success (Sardans et al., 2011), or at least allow to highlight some specificities of invasive species. For instance, Macel et al. (2014) showed that invasive species of Asteraceae have different and more specific metabolic profiles compared to native species. Comparative responses of native and invasive aquatic plants to increased temperatures have been investigated in a few studies (McKee et al., 2002; Mormul et al., 2012). However, to our knowledge only two studies have combined both morphological trait approaches and metabolic profiling on aquatic plants in order to evaluate an integrated response to abiotic factors (Hussner et al., 2016; Thouvenot et al., 2015). Moreover, according to Peñuelas *et al.* (2004), plant processes can be more or less sensitive to warming depending on the season, and Silveira and Thiébaut (2017) highlighted that for submersed plant species, a rise of temperature had a greater impact on plant morphological traits in spring than in summer.

In the present study, we used this combined approach to test the effects of an experimental warming of spring and summer mean temperatures on growth and carbon metabolism of four amphibious species. We hypothesised that (i) a 3°C warming induces changes on plant morphology and qualitative and/or quantitative modifications of carbohydrate metabolism, (ii) there are more changes due to this temperature increase in spring than in summer, (iii) the induced modifications are different between native and invasive species.

2. Materials and methods

2.1.Studied species

Four amphibious species were chosen, two species invasive in Europe (*Ludwigia hexapetala*, *Myriophyllum aquaticum*), and two other species native to Europe (*Mentha aquatica*, *Myosotis scorpioides*). Some characteristics of these species are summarised in Table 1.

2.2.Temperature settings

Temperature settings were based on the maximal monthly temperature (1981-2010) recorded in north western France (data source: Meteo France). The average maximal temperature calculated is 15.7°C in spring (March, April, May) and 23°C in summer (June, July, August). In our experiments, we applied a 3°C warming above current temperatures, based on the future IPCC temperature projections. Thus, four temperatures were selected for two experiments. Experiment 1: 16°C (average spring temperature), 19°C (3°C above average maximal spring temperatures). Experiment 2: 23°C (average summer temperature) and 26°C (3°C above average maximal summer temperatures).

2.3.Experimental design

Young plant of *L. hexapetala*, *M. aquaticum* (herafter *M. brasiliense*), *M. aquatica*, and *M. scorpioides* at the same developmental stage were collected in spring (experiment 1) and summer (experiment 2) from the same pond in Brittany, France (48°05’31.3’’N; 01°44’41.3’’W), probably from the same individual. The experiments were conducted in April and June 2014, respectively. Plants were acclimatised for a week in tap water at room temperature. For each species, 20 shoots with apices were cut to 10 cm lengths. Shoots had no buds or lateral stems. Each shoot was planted in individual containers (L x W x H: 8 x 8 x 22 cm) filled with a substrate composed of 1 cm of loam (NPK 16-7-15) and 1 cm of sand. Containers were filled with 150 mL of tap water i.e. 1 cm above substrate. Tap water had a moderate nitrogen concentration, low phosphates, high nitrates and was slightly basic

(average values for tap water: conductivity = 435 µS.cm^{-1}; pH 8.1 ; [NO_3^-] = 32.7 mg L^{-1}; [PO_4^{3-}] < 0.02 mg.L^{-1}; [NO_2^-] < 0.02 mg.L^{-1}; [NH_4^+] < 0.03 mg.L^{-1}).

Containers were randomly distributed in two different growth chambers (Percival AR-41L3X) programmed with the chosen temperatures (experiment 1: spring temperatures, 16°C and 19°C; experiment 2: summer temperatures, 23°C and 26°C). Within the chambers, light intensity was about 330 µmol.m^{-2}.s^{-1}, with a 12:12 photoperiod, and relative humidity was maintained at 75%. Plants were exposed to these conditions for 10 days.

2.4.Morphological traits

We measured four morphological traits at the beginning and at the end of the experiment: stem length, fresh weight, number of lateral shoots (buds and ramification) and number of roots. These four traits are linked to functions, the apical growth, the vigour, the lateral growth and the root production, respectively. For each experiment, fresh weight measurements were carried out immediately after removing and cleaning plants from the substrate, and the excess water was blot using paper towel. We considered that plant fresh weight is a global measurement which depict the multiplication or extension of cells, leading either to apical growth, lateral growth, root production, but also to modification of tissue thickness. Thus, we linked plant fresh weight to its vigour. According to Hunt (2003), a relative growth rate (RGR; d^{-1}) can be calculated with trait measurements such as length or weight. In this study, calculations for stem and weight RGR were adapted from the RGR calculation of Hunt (1990):

$$RGR\ stem = (\ln L2 - \ln L1)/(T2 - T1)$$

where L1 and L2 represent total length , at time T1 (beginning of the experiment) and T2 (end of the experiment).

$$RGR\ weight = (\ln M2 - \ln M1)/(T2 - T1)$$

where M1 and M2 represent the plant's fresh mass respectively at time T1 and T2.

2.5.Carbohydrate analysis

At the end of the experiment, the upper five centimetres of each plant were cut. Nine of these apical parts were pooled by species and by temperature conditions by group of three, resulting in three samples per temperature condition for each species. Plant fragments were frozen in liquid nitrogen, stored at -80°C, lyophilised and reduced into powder. 10 mg of plant powder were used for each sample, polar components were extracted by a methanol-chloroform-water extraction, and carbohydrates were quantified by GC-MS as described by Thouvenot *et al.* (2015). 400 µL of methanol containing an internal standard (20 mM ribitol), 200 µL of chloroform and 400 µL of water were added successively, after 15 minutes of agitation between each addition. Samples were centrifuged for 5 min at 13 000 g, 4°C. The chloroform phase was discarded, and 50 µL of the methanol:water phase was dried using a SpeedVac system. Dried extracts were stored at -20°C until analysis. For GC-MS analysis, vacuum-dried phase aliquots were re-suspended in 50 µL of methoxyaminehydrochloride solution in pyridine (20 $mg.mL^{-1}$). Samples were agitated for 90 min at 30°C, and 50 µL of N-methyl-N-(trimethylsilyl)trifluoroacetamide were then added; derivatisation was conducted at 37°C for 30 min under agitation. Samples were then transferred into glass vials and incubated at room temperature over-night before injection. Metabolite content was estimated in reference to the ribitol signal and dry weight of samples.

2.6.Statistical analyses

Statistical analyses were performed using statistical R 3.2.3 software (R Development Core Team, 2015) with packages multcompView and ade4 (Dray and Dufour, 2007). Plots were generated using packages ggplot2 (Wickham, 2009) and factoextra (Kassambara and Mundt, 2016). A two way ANOVA was performed to test the effect of species, temperature and their interaction on functional traits. Pairwise post-hoc comparisons were performed with Tukey HSD test. Normality and homogeneity of variances were tested prior to each parametric test, and data were transformed if necessary by square root or 1/x transformation in order to meet statistical model assumptions.

We aim at using metabolic profiles to infer physiological patterns of response. However, it is difficult to derive physiological information due to the high number of carbohydrates involved (n≥20 in this study). Moreover, part of the information is expected to be redundant as carbohydrates involved in same physiological processes can be interrelated. Methods of dimensionality reduction (Legendre and Legendre, 1998) allow compressing data down to a few important axes of variation (greatly reducing the number of parameters of interest) and help visualising inherent patterns in the dataset. Those methods are commonly used in vegetation ecology where they are applied on data from floristic surveys to derive the environmental gradients underlying plant species distribution (ter Braak and van Tongeren, 1995; Wittaker and Gauch, 1982). Here, we performed a Principal Component Analysis (PCA) on species x carbohydrate content matrix for each temperature regime tested (experiments 1 and 2). Then we examined whether species scores on the first and second PCA axis could be used as a synthetic variable related to species response to temperature. Because in both experiments the first axis mainly represents a species effect, we have chosen to use the second axis as a synthetic variable related to species response to temperature. A two ways ANOVA was finally performed on the mean coordinates of individuals on the selected synthetic variable to test the crossed effects of species and temperature.

We also performed Student's t-tests on total and individual contents in soluble sugars and organic acids, by species, in order to test for quantitative differences induced by temperature treatments.

3. Results

3.1.Effects of a 3°C increase of spring temperature (experiment 1)

3.1.1. Morphological traits

Apical and lateral growth varied according to species and temperature interaction (Table 2). Indeed, the two exotic species *M. brasiliense* and *L. hexapetala* had a significantly higher apical growth at 19°C than at 16°C (Fig. 1A, B), and *L. hexapetala* was the only species that produced significantly more lateral shoots in response to the 3°C warming, although *M. aquatica* also increased its

production of lateral shoots with the temperature warming (Table 2). Regardless of the species, the vigour and the root production were higher at 19°C than at 16°C (Table 2).

Our results showed that the growth strategies of the four species were all different (Fig. 1). Regardless of temperature, both native species *M. aquatica* and *M. scorpioides* seemed to favour root production over apical growth, unlike invasive species for which apical growth was more important than root production (Fig. 1C, D). However, *M. scorpioides* had the highest vigour at 19°C, while *M. aquatica* had the lowest, and conversely *M. aquatica* had the highest lateral growth whereas *M. scorpioides* had the lowest at 19°C (Table 2). The lateral growth of *M. brasiliense* was lower than those of *M. aquatica* at 16°C, and the number of lateral shoots of *M. scorpioides* and *M. brasiliense* was lower than those of *M. aquatica* and *L. hexapetala* at 19°C.

3.1.2. Metabolic profiles

The PCA showed that 56.7% of the carbohydrates were explained by the two first axis (Fig. 2A, B). The contribution of the variables to these axes can be seen in Fig. S1A (Supporting information). A clear opposition was established by the first axis between *M. aquatica* and the three other species (Fig 2A), due to the presence of compounds uniquely in *M. aquatica*, and of compounds present at 16°C and/or 19°C in higher concentrations in this species than in the three others (see Table S1). Scores on axis 2 allow the discrimination of the four plant species and appeared also to be sensitive to temperature (Fig. 2B). The ANOVA showed that there was a significant interaction between species and temperature (Table 4). Interestingly, it appeared that the overall composition in carbohydrates was significantly modified by a 3°C warming in spring only for the two native species (Fig. 2E). Thus, native and invasive species were discriminated by two different sets of carbohydrates and the contents of carbohydrates specific to native species tend to increase with higher temperature.

The soluble sugars content (glucose, fructose, saccharose…) was slightly affected by the warming, except for *M. brasiliense* for which it was significantly lower at 19°C (Fig. 3A). However, it appeared

that when considered individually, the content of some soluble sugars was modified by the temperature treatment: content in fructose decreased for *M. aquatica*, while the content in sucrose increased for *M. scorpioides* at 19°C (Table 5). The content in organic acids, notably of those involved in photorespiration (glycerate, glyoxylate…), increased with the 3°C warming in spring only for *L. hexapetala* (Fig. 3B), concomitant with the increase in malate and glycerate content (Table 5). Content in carbohydrates related to raffinose family oligosaccharides (RFO) metabolism (raffinose, galactinol, myo-inositol...) were significantly lower at 19°C than at 16°C for both invasive species (Fig. 3C). This can be explained by the significantly decrease in galactinol and myo-inositol for *L. hexapetala* and *M. brasiliense* respectively (Table 5). *M. aquatica* presented a RFO content 3-fold higher than for the other species (Fig. 3C), due to high contents in raffinose, galactose and galactinol.

3.2.Effects of a 3°C increase of summer temperature (experiment 2)

3.2.1. Morphological traits

There was no effect of the 3°C warming of summer temperatures on the measured functional traits (Table 3). However, there was significant differences between species for the four traits ($P<0.001$, Table 3), independently of temperatures. *M. aquatica* had a low apical growth, a low vigour, the highest root production, and high lateral growth. *M. scorpioides* was characterised by a low apical growth, high vigour, a moderate production of roots, and high lateral growth (Fig. 1). At tested temperatures, *L. hexapetala* had a high apical growth, a moderate vigour, low production of roots, and high lateral growth. *M. brasiliense* characteristics were a high apical growth, high vigour, low root production, and the lowest lateral growth.

3.2.2. Metabolic profiles

The PCA showed that the two first principal components accounted for 71.8% of the total variance in carbohydrates content (Fig. 2C, D). The contribution of the variables to these axes can be seen in Fig. S1b. The first axis separated *M. scorpioides* from the three other species (Fig 2C), which is mainly explained by its high content in sucrose, quinate and malate compared to the three other species, as

well as the presence of glycolate and cellobiose only in this species (Table S2). However, the PCA showed no separation of individuals by temperature (Fig. 2B). The ANOVA performed confirms that there was no temperature effect, but a strong species effect with a clear discrimination between native and invasive species (Fig 2F, Table 4), invasive species being rather characterised by glycerate and fructose, which were less abundant in native species.

The warming led to a decrease in total soluble sugars content only for the two invasive species (Fig. 3D), explained by a drastic decrease in fructose and glucose content for these species at 26°C (Table 5). Otherwise, the temperature increase had no impact on the content in soluble sugars for the two native species (Fig. 3D). Unlike to the warming in spring, the temperature increase in summer had no effect neither on the content in organic acids nor on the content in RFO-related carbohydrates, whatever the species (Fig. 3E, F; Table 5).

4. Discussion

The aim of this study was to investigate the impact of a 3°C warming in spring and in summer on two invasive (*L. hexapetala*, *M. brasiliense*) and two native species (*M. aquatica*, *M. scorpioides*). We focused on the changes induced by temperature increase on four morphological traits and on carbohydrates content. Primary metabolism to highlight , but also differences between the metabolism or the metabolites composition of invasive and native species, as shown by Macel et al 2014 or Funk et al 2011. We focused on carbohydrates for some of the studied species, these compounds has been shown to react to other abiotic factors (Thouvenot et al). Investigating the metabolic profiles of invasive and native species can allow to better understand the invasiveness of aquatic plant species, and the possible impact/response to future environmental changes.

4.1.Temperature and seasonal effects

The growth of both native and invasive species was enhanced by a 3°C warming in spring, but none of them was morphologically affected by an increase of summer temperature. Likewise, the quantitative synthetic response of carbohydrates was affected only in spring, especially for the native

species. However, detailed results focused on single soluble sugar contents showed that they were not only modified in spring, but also in summer, depending on species and on type of carbohydrates. Thus, our first hypothesis in partially validated, a 3°C warming induced changes in plant morphology and/or qualitative or quantitative modifications of carbohydrate metabolism.

The qualitative approach showed that the warming applied in spring induced changes in carbohydrate profiles in native species, though changes in detected carbohydrates were mainly due to low carbohydrate contents. The decrease in fructose and glucose contents in *M. brasiliense* when exposed to a 3°C warming in spring may reflect a direct allocation of photosynthetates to apices growth more effective at 19°C than at 16°C, an assumption supported by morphological measurements. Surprisingly, except for *M. scorpioides,* sucrose content was not significantly modified at 19°C in the apices of the studied species whereas sucrose is the major transport form of organic carbon in most plants. As an exemple, Havelange *et al.* (2000) showed that in *Sinapis alba*, an increase of sucrose content in roots was related to a rise in the amount of sucrose exported by young leaves. Furthermore, it is known that the partitioning of photosynthetates between starch and sugars and is influenced by environmental factors such as temperature, leading to changes in sugars content. For example, Lafta and Lorenzen (1995) showed that in *Solanum tuberosum*, high temperature induced sucrose accumulation coinciding with decreased starch content in leaves without a change in glucose content. Similar results were observed in our experiment for *M. scorpioides* for which sucrose accumulated at 19°C whereas fructose and glucose content was strictly identical at 16°C and 19°C, suggesting that for this species photoassimilates were partitioning.

The higher malate content of *L. hexapetala* at 19°C may indicate a stimulation of photosynthetic activity in this C4 species (Madanes et al., 2015) by temperature warming. Photoassimilates may be used for apical and lateral growth, which would be consistent with the observed increase of these morphological traits at 19°C. Indeed, *L. hexapetala* was the only species which had both high apical and lateral growth, and is the only one using a C4 photosynthetic

pathway. Thus, our results suggest that *L. hexapetala* could be classified as a "malate former" among C4 species, with malate being the predominant initial product of photosynthesis (Hatch and Boardman, 2014), which seems to be the case of other C4 macrophytes species as well (Beer and Wetzel, 1982; Bowes et al., 2002). According to Dukes and Mooney (1999) and Sage and Kubien (2007), C4 species might benefit more from warmer temperature than C3 species because of their generally higher optimum temperature for photosynthesis. This suggests that the colonisation by *L. hexapetala* may particularly be favoured by climate warming.

Surprisingly, the content in RFO-related carbohydrates decreased only for both invasive species in response to spring warming. These metabolites accumulate in response to a range of abiotic stress, notably in response to cold stress, in which raffinose might have a protecting effect on chloroplast (Findling et al., 2015). We can hypothesize that 16°C could be a sub-optimal temperature for these species and so be perceived as a slight stress. In this case, as at 19°C RFO metabolism would be less requested, it can be expected that carbohydrates required for RFO synthesis may be redirected towards biomass production. This could explain the highest apical growth observed for both invasive species at 19°C.

Despite an unchanged qualitative composition in carbohydrates and an absence of differences in plant growth, the increase of 3°C in summer led to a strong decrease in fructose and glucose contents in both invasive species *L. hexapetala* and *M. brasiliense* whereas sucrose content was not modified. If we consider that, as observed for a spring warming, a summer warming stimulates photosynthesis of the invasive species, beneficial effect on carbohydrates production do not necessarily result in greater growth. We can hypothesise that the excess of photoassimilates produced by a more efficient photosynthesis might be allocated to energy storage in starch and/or carbon storage in storage organs. Indeed, recent studies showed that starch content in *M. brasiliense* was higher in summer than in spring and was preferentially stored in stolons (Wersal et al., 2011).

Moreover such modification of the carbon partitioning is also a common feature of environmental stress (Rosa et al., 2009).

The present study established that a 3°C warming had higher impact on growth and metabolism in spring than in summer, a result consistent with our second hypothesis. This result was expected as spring is the season when these species allocate most of their energy to apical or lateral growth and to nutrients uptake, in order to compete with other species and to survive. In summer they also allocate their energy to sexual reproduction (Stiers et al., 2014; Thouvenot et al., 2013). Moreover, an increase of 3°C did not have the same consequences on metabolism at 16°C as at 23°C: differences in enzyme activities are susceptible to be more important from 16°C to 19°C than from 23°C to 26°C. The effect of an increase of temperature on plant growth will depend on the period of the year where the warming occurs. It would be interesting to study the impact of a temperature increase on the whole plant life cycle, in order to establish if modifications occurring in spring have an after effect on species spread.

4.2.Species effect

Our results showed a strong species effect in both experiments, regardless of the applied temperature. Contrary to the findings of van Kleunen *et al.* (2010), our results showed that invasive species did not have higher values than native species for traits related to performance. Indeed, regardless of the temperature effect on their growth, the four species developed different growth strategies. Both invasive species favoured apical growth whereas native species allocated energy to the production of roots. Thus, *M. aquatica* and *M. scorpioides* could stay rooted in sediment during flooding events, but may also have a better access to water and nutrients from soil during drawdown, as macrophytes are able to acquire nutrients from both the sediment and water column (Bristow and Whitcombe, 1971; Rattray et al., 1991). In contrast, the low production of roots of *L. hexapetala* and *M. brasiliense* may confer upon them the ability to disperse more easily through water flow, and their high apical growth allows them to outcompete other species for light

acquisition. Thus, the studied native and invasive species seem to present a trade-off between their regeneration (apical growth) and their colonisation (root production) abilities (Barrat-Segretain et al., 1998). However, lateral growth and general vigour were different within invasive and native species. The high lateral growth of *M. aquatica* could allow individuals to escape from the shadow created by other species. The four species live in the same ecosystems, but each of them adopts its own growth strategy to face competition, resource acquisition and climate events.

The morphological results observed for the two invasive species are consistent with the work of Rejmánková (1992), who showed that creeping emergent species such as *Ludwigia peploides* and *M. brasiliense* are characterised by rapid growth and allocation of most biomass and nitrogen into aboveground plant parts. Additionally, invasive species tended to have the same patterns of carbohydrates content in their apical parts, whether in spring or in summer. These invasive species seems to have common physiological characteristics, that might result from a common adaptation to local climate through the selective pressure generated by the invasion process (Lee, 2002), and that favour their high and rapid growth.

As there was no clearly pronounced differences between exotic and native species, we our third hypothesis cannot be confirmed with certainty. Literature data suggest that global warming might induce the dominance of invasive species over natives (Netten et al., 2010), but our results are consistent with this statement only for *L. hexapetala*, and mostly when the warming occurs in spring. To establish if invasive species would be dominant over native species require to grow them in mixture, especially because allelopathic effects are suspected for *L. hexapetala* (Dandelot et al., 2008) and *M. aquatica* (Lovett and Weerakoon, 1983).

In this study, the carbohydrates profiling was performed on apical parts of plants, which allows us mainly to link the carbohydrates profiles to apical growth. Metabolic profiling analysis at the level of the other plant organs than that we studied will be necessary in order to confirm our assumptions on carbon allocation, especially analysis of carbohydrate contents in roots and lateral branches,

associated with the determination of starch content in the different organs in spring and in summer. Profiling other metabolites or focusing more specifically on photosynthesis could also bring more information and allow a better understanding of the studied species response to climate warming. Moreover, temperature is not the only factor that could influence future species distribution, and the combination of multiple component of climate change (alteration of water chemistry and hydrological regimes) could also be studied.

5. Conclusion

Our study showed that the impact of a 3°C warming on four aquatic plants species were more intense in spring than in summer. In spring, the apical and lateral growth of the two invasive species was particularly affected by a temperature increase. The warming globally improved the vigour and the production of roots of the four freshwater species in spring. Invasive species metabolism was stimulated by a 3°C warming in summer, but with no effect on their morphological characteristics. Climate warming might favour the growth of invasive species over native species, especially during spring, with possible repercussion on the entire growing season as well as on plant community composition and on ecosystem functioning.

No difference between invasive and native species can be made with respect to the growth measurement results. However, patterns of contents in carbohydrates group the two invasive species together both in spring and in summer experiments, possibly highlighting common physiological mechanisms explaining their invasiveness. Of the four studied species, *L. hexapetala* had the most effective apical and lateral growth. Its C4 photosynthesis seems to confer on it an advantage to colonise water bodies. Although apical and lateral growth of *M. scorpioides* was not enhanced by warming in spring, its high capacity to produce carbohydrates such as sucrose, associated with its increased vigour could allow it to take advantage from climate warming.

This study is a first sketch about the impacts of rising temperatures on the metabolome of native and invasive macrophytes. The use of metabolic profiling to study responses of plant species with no-

agronomic interest is at its infancy. We showed that this approach reveals differential responses among species, which could be a clue to identify potential mechanisms of invasiveness of alien species. Further investigation about amino acid profiling, gene expression or enzyme activity of invasive plant species could be of broad interest to understand the mechanisms behind their strong proliferation in their exotic ranges, and to study response to biotic and abiotic factors.

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Authors' contribution

MG, CD and GT designed the experiment. MG conducted the experiments. MG and CD performed the extraction of polar components. SB performed the GC-MS analyses. MG, CD and NR analysed the data. MG and CD interpreted the data and wrote the manuscript with contributions from all the authors.

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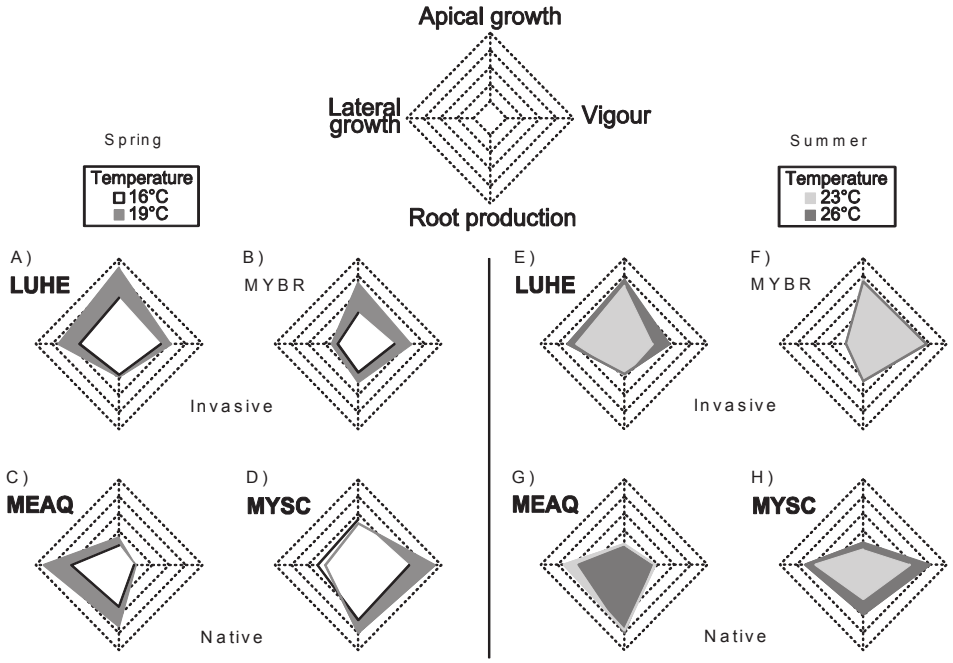
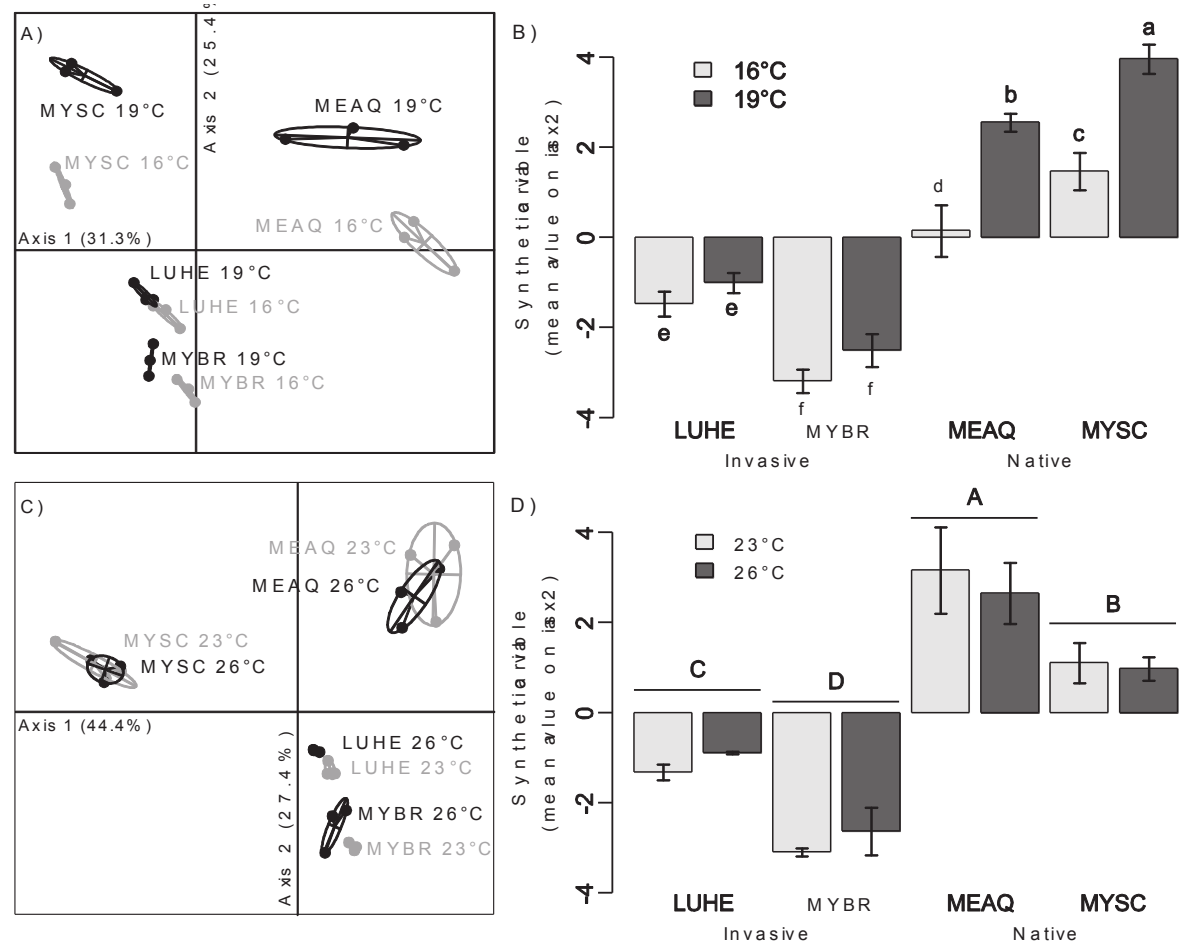
Figure legend

Fig. 1. Functional responses of *Ludwigia hexapetala* (LUHE), *Myriophyllum brasiliense* (MYBR), *Mentha aquatica* (MEAQ) *and Myosotis scorpioides* (MYSC) to a 3°C warming of a spring temperature (A,B,C,D) and of a summer temperature (E,F,G,H). Values represented have been normalised per trait

Fig. 2. Principal component analysis (PCA) on carbohydrate content with a 3°C increase of spring temperature (A,B) and of summer temperature (C,D) for *Ludwigia hexapetala* (LUHE,), *Myriophyllum brasiliense* (MYBR), *Mentha aquatica* (MEAQ,) and *Myosotis scorpioides* (MYSC). Individuals were grouped by species (A,C) and by temperature (B,D). On panels B and D, dots and lines in light grey represent the control temperature (16°C or 23°C) and those in black represent the warmer temperature (19°C of 26°C). Lines link individuals to their respective centroids (n=6, A,C; n=24, B,D). Refer to Fig. S1 for correlation circles. Barplots in panel (E) and (F) represent a synthetic representation of the qualitative modifications in carbohydrate content depending on temperature, i.e. the mean coordinates of individuals on Axis 2 ± SE, at both seasons. Different small letters indicate significantly different interactions between species and temperature (E). Different capital letters represent significant differences between species (F).

Fig. 3. Total content in soluble sugars, in organic acids and in Raffinose Family Oligosaccharides (RFO) (±SE) of *Ludwigia hexapetala* (LUHE,), *Myriophyllum brasiliense* (MYBR), *Mentha aquatica* (MEAQ,) and *Myosotis scorpioides* (MYSC). at 16°C and 19°C (A,B,C) and at 23°C and 26°C (D,E,F). Stars indicate significant differences between temperature regimes, Student's t-test: * p<0.05, ** p<0.01, *** p<0.001





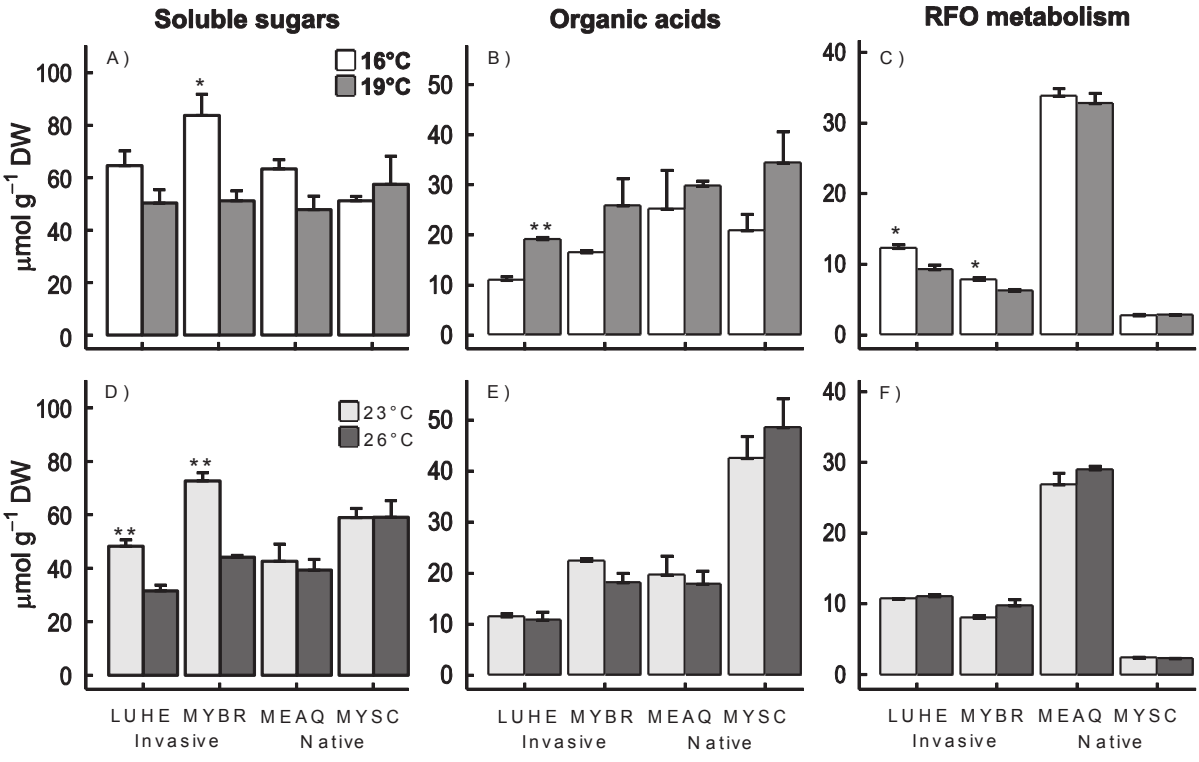


Table 1. Informations about the four amphibious species studied.

Species	*Ludwigia hexapetala* (Hook. & Arn.) Zardini, H.Y. Gu & P.H. Raven	*Myriophyllum aquaticum* (Vell.) Verdc	*Mentha aquatica* L.	*Myosotis scorpioides* L.
Synonym	*Ludwigia grandiflora* subsp. *hexapetala*; *Ludwigia uruguayensis*	*Myriophyllum brasiliense*	*Mentha hirsuta*	*Myosotis palustris*
Common name	Waterprimrose	Parrotfeather	Water mint	Water forget-me-not
Family	Onagraceae	Halogaraceae	Lamiaceae	Boraginaceae
Native to	South America	South America	Europe	Europe
Introduced or invasive in	Europe, North america, Australia, New Zealand, Turkey (Thouvenot et al., 2013)	Europe, North america (Hussner et al., 2009; Thiébaut, 2007)	North America (USDA, 2017)	North America (USDA, 2014)
Photosynthetic pathway	C4 (Madanes et al., 2015)	C3	C3	C3
Temperature requirements	Minimum temperature to initiate growth : 12°C to 15°C (EPPO, 2011) ; optimum development between 20°C and 30°C (Ruaux, 2008)	Growth begin when water temperature reach 8°C (Moreira et al., 1999)	N.A.	N.A.

Table 2. Summary of ANOVA on morphological traits and transformed traits to a 10-day exposition to spring temperature (16°C and 19°C). Different capital letters represent significant differences between species. Different small letters indicate significantly different interactions between species and temperature. Different capital letters represent differences between species. The symbol * highlights an overall temperature effect. ns = not significant (P<0.05, Tukey HSD test). Effect of species: n= 40, df=3 ; effect of temperature: n= 80, df= 1; effect of species x temperature interaction: n=20, df=3.

Traits	Effect	% sum sq	F value	P value	Tukey HSD	
RGR stem	Species	28.7	22.56	<0.001		
	Temperature	8.6	20.20	<0.001		
	Species x temperature	4.9	3.84	0.011	*L. hexapetala* 16°C	bc
					L. hexapetala 19°C	a
					M. aquatica 16°C	e
					M. aquatica 19°C	de
					M. brasiliense 16°C	cde
					M. brasiliense 19°C	ab
					M. scorpioides 16°C	bcd
					M. scorpioides 19°C	bcd
	Residuals	57.8				
RGR fresh weight	Species	43.2	40.98	<0.001	*L. hexapetala*	B
					M. aquatica	C
					M. brasiliense	B
					M. scorpioides	A

	Temperature	5.0	14.35	<0.001	16°C	
					19°C	*
	Species x temperature	1.9	1.83	ns		
	Residuals	49.9				
Number of roots	Species	21.9	14.18	<0.001	*L. hexapetala*	B
					M. aquatica	A
					M. brasiliense	B
					M. scorpioides	A
	Temperature	3.0	5.92	0.016	16°C	
					19°C	*
	Species x temperature	0.9	0.56	ns		
	Residuals	74.2				
Number of lateral shoots	Species	26.3	19.75	<0.001		
	Temperature	3.5	7.84	0.006		
	Species x temperature	6.3	4.77	0.003	*L. hexapetala* 16°C	c
					L. hexapetala 19°C	ab
					M. aquatica 16°C	abc
					M. aquatica 19°C	a
					M. brasiliense 16°C	d
					M. brasiliense 19°C	cd
					M. scorpioides 16°C	bc
					M. scorpioides 19°C	cd
	Residuals	63.9				

Table 3. Summary of ANOVA on morphological traits and transformed traits to a 10-day exposition to summer temperature (23°C and 26°C). Different capital letters represent significant differences between species. Different small letters indicate significantly different interaction between species and temperature. Different capital letters represent differences between species. The symbol * highlights an overall temperature effect. ns = not significant (P<0.05, Tukey HSD test). Effect of

species: n= 40, df=3 ; effect of temperature: n= 80, df= 1; effect of species x temperature interaction: n=20, df=3.

Traits	Effect	% sum sq	F value	P value	Tukey HSD	
RGR stem	Species	60.6	61.69	<0.001	*L. hexapetala*	A
					M. aquatica	B
					M. brasiliense	A
					M. scorpioides	B
	Temperature	0.1	0.32	ns		
	Species x temperature	0.6	0.58	ns		
	Residuals	38.7				
RGR fresh weight	Species	25.9	14.46	<0.001	*L. hexapetala*	B
					M. aquatica	B
					M. brasiliense	A
					M. scorpioides	A
	Temperature	1.9	3.26	ns		
	Species x temperature	2.9	1.59	ns		
	Residuals	69.3				
Number of roots	Species	27.4	15.28	<0.001	*L. hexapetala*	B
					M. aquatica	A
					M. brasiliense	B
					M. scorpioides	B
	Temperature	0.4	0.63	ns		
	Species x temperature	0.9	0.51	ns		
	Residuals	71.3				
Number of lateral shoots	Species	34.09	21.63	<0.001	*L. hexapetala*	A
					M. aquatica	A
					M. brasiliense	B
					M. scorpioides	A
	Temperature	0.02	0.04	ns		
	Species x temperature	3.40	2.15	ns		
	Residuals	62.49				

Table 4. Summary of ANOVA on synthetic values of carbohydrate content at 16-19°C and at 23-26°C.

Traits	Effect	df	% sum sq	F value	P value
Synthetic variable	Species	3	84.34	309.66	<0.001
16-19°C	Temperature	1	10.26	112.99	<0.001
	Species x temperature	3	3.95	14.52	<0.001
	Residuals		1.45		
Synthetic variable	Species	3	95.83	0.09	<0.001
23-26°C					
	Temperature	1	0.02	154.31	ns
	Species x temperature	3	0.84	1.35	ns
	Residuals		3.31		

Table 5. Individual soluble sugar content (±SE) in *Ludwigia hexapetala*, *Myriophyllum brasiliense*, *Mentha aquatica* and *Myosotis scorpioides,* significantly different between 16°C and 19°C and between 23°C and 26°C. (A) soluble sugars; (B) organic acids; (C) RFO metabolism. nd=not detected

Species	Temp	Fructose (A)	Glucose (A)	Sucrose (A)	Malate (B)	Glycerate (B)	Myo-inositol (C)	Galactinol (C)
Ludwigia	16°C	28.53 ± 3.79	15.44 ± 2.92	19.23 ± 2.07	6.69 ± 0.59	1.04± 0.1	5.30 ± 0.08	4.49 ± 0.29
hexapetala	19°C	19.82 ± 1.86	11.54 ± 2.12	17.75 ± 1.81	13.9 ± 0.47 ***	1.57± 0.14 *	4.97 ± 0.29	2.53 ± 0.21 **
Myriophyllum	16°C	43.66 ± 4.89	17.37 ± 3.76	19.04 ± 1.53	7.57± 0.67	4.69± 0.42	7.35 ± 0.34	0.04 ± 0.04
brasiliense	19°C	24.98 ± 0.98	11.02 ± 1.09 ***	13.17 ± 1.84	10.01± 2.12	10.8± 3.07	5.76 ± 0.23 *	0.03 ± 0.03
Mentha	16°C	18.92 ± 1.48	25.08 ± 2.71	14.14 ± 2.31	9.96± 4.6	nd	5.18 ± 0.23	7.72 ± 0.27
aquatica	19°C	12.30 ± 1.04 *	17.80 ± 6.74	16.39 ± 0.89	15.2± 3.38	nd	5.99 ± 0.92	8.13 ± 0.67
Myosotis	16°C	11.35 ± 0.77	8.14 ± 1.72	29.87 ± 0.77	5.59± 1.23	0.47± 0.23	2.69 ± 0.21	nd
scorpioides	19°C	11.24 ± 6.88	8.25 ± 5.12	37.24 ± 0.39 ***	16.38± 6.06	1.04± 0.27	2.83 ± 0.06	nd
Ludwigia	23°C	18.09 ± 1.78	9.77 ± 0.26	19.95 ± 0.91	8.05± 0.45	1.67± 0.27	4.77 ± 0.09	2.62 ± 0.16
hexapetala	26°C	6.34 ± 0.95 **	4.62 ± 0.59 ***	20.18 ± 0.66	8.00± 1.41	0.81± 0.07	4.33 ± 0.29	2.73 ± 0.004

Myriophyllum	23°C	41.02 ± 2.19	15.83 ± 1.13	14.94 ± 0.24	10.68± 0.46	8.34± 0.25	7.03 ± 0.15	0.32 ± 0.10
brasiliense	26°C	22.10 ± 0.97 ***	7.82 ± 0.27 **	13.75 ± 0.97	9.06± 0.62	6.30± 1.75	6.92 ± 0.35	1.22 ± 0.32
Mentha	23°C	10.62 ± 1.61	18.02 ± 4.13	13.00 ± 1.01	8.99± 1.92	nd	4.86 ± 0.42	5.86 ± 0.61
aquatica	26°C	8.80 ± 1.06	16.85 ± 2.49	13.12 ± 0.51	7.01± 0.80	nd	4.86 ± 0.27	6.04 ± 0.70
Myosotis	23°C	6.37 ± 0.84	6.33 ± 0.43	45.40 ± 4.50	17.56± 1.83	0.59± 0.11	2.44 ± 0.13	0.01 ± 0.00
scorpioides	26°C	8.49 ± 2.38	7.23 ± 1.79	42.31 ± 2.44	26.37± 4.78	0.75± 0.30	2.19 ± 0.19	0.035 ± 0.03