Accepted: 16 April 2018

DOI: 10.1111/fwb.13124

ORIGINAL ARTICLE

WILEY Freshwater Biology

Seasonal dependence and functional implications of macrophyte–phytoplankton allelopathic interactions

Mathieu Santonja | Bertrand Le Rouzic | Gabrielle Thiébaut

CNRS, ECOBIO UMR 6553, Univ Rennes, Rennes, France

Correspondence
Mathieu Santonja, CNRS, ECOBIO UMR 6553, Univ Rennes, Rennes, France.
Email: mathieu.santonja@gmail.com

Funding information
ECOBIO - Axe "Biological Invasions"

Abstract

Invasive plant species such as *Ludwigia hexapetala* might have a competitive advantage if they produce allelopathically active compounds against primary producers. Both phytoplankton and plant community structure may be affected due to different, species-specific sensitivity to allelochemicals. Moreover, such allelopathic interactions could vary over the year depending on (i) the plant's phenological stage and (ii) the abilities of the native macrophytes to suppress—or the non-native macrophytes to stimulate—the non-native macrophyte population. We tested the allelopathic effects of aqueous leaf extracts of *L. hexapetala* on the photosynthetic activity of three target phytoplankton strains (*Scenedesmus communis*, a toxic *Microcystis aeruginosa* strain and a non-toxic *Microcystis aeruginosa* strain) over three seasons of development (spring, summer and autumn). We also tested seasonal allelopathic effects of aqueous leaf extracts of both *L. hexapetala* (i.e. the non-native invasive species) and the native *Mentha aquatica* on *L. hexapetala* seed germination. Finally, we identified three main secondary compounds present in the aqueous leaf extracts of *L. hexapetala* and we tested each individual compound on the phytoplankton's photosynthetic activity and on *L. hexapetala* seed germination. We observed marked seasonal and species-specific patterns of *L. hexapetala* allelopathy on phytoplankton. The photosynthetic activities of *S. communis* and the toxic *M. aeruginosa* strain were stimulated by *L. hexapetala* aqueous leaf extracts in autumn and spring, respectively, whereas the non-toxic *M. aeruginosa* strain was strongly inhibited in these two seasons. In summer, photosynthesis of all phytoplankton strains was inhibited. The germination rate of *L. hexapetala* seeds was stimulated by both *L. hexapetala* and *M. aquatica* aqueous leaf extracts, especially in summer, concomitant with the strong negative effects observed on the three phytoplankton strains. Three flavonoid glycosides (myricitrin, prunin and quercitrin) were identified as the main secondary compounds present in the *L. hexapetala* aqueous leaf extracts. The photosynthetic activity of *S. communis* was slightly stimulated by the three compounds. The photosynthetic activity of the toxic *M. aeruginosa* strain was stimulated by myricitrin and quercitrin, whereas that of the non-toxic *M. aeruginosa* strain was inhibited by prunin. Finally, the germination rate and the germination velocity of *L. hexapetala* seeds were stimulated by myricitrin and prunin. These findings suggest that *L. hexapetala* could favour the photosynthetic activity of toxic cyanobacteria in spring and reduce their photosynthetic activity in summer, potentially leading to drastic changes in the phytoplankton communities and therewith

ecological functioning of invaded ponds. Moreover, the stimulation of its seed germination could give a strong competitive advantage to *L. hexapetala*, thus promoting its invasiveness.

KEYWORDS
allelochemicals, aquatic ecosystems, biological invasion, cyanobacteria, *Ludwigia hexapetala*

1 | INTRODUCTION

The outcome of the competition between macrophytes and phytoplankton for light, carbon and nutrients has important consequences for the functioning of aquatic ecosystems (Gross, 2003; Scheffer, Hosper, Meijer, Moss, & Jeppesen, 1993). Macrophyte-dominated systems generally tend to have good water quality and high biodiversity, while phytoplankton-dominated systems are more often associated with low biodiversity and poor water quality (Declerck, Bakker, van Lith, Kersbergen, & van Donk, 2011). Macrophytes can stimulate changes to clear water situations through a range of mechanisms (van Donk & van de Bund, 2002). One of the possible ways through which macrophytes control phytoplankton is by producing secondary compounds (allelochemicals) which reduce phytoplankton growth (Fleming & Dibble, 2015; Gross, 2003; Hilt & Gross, 2008; Mulderij, Mau, van Donk, & Gross, 2007). However, phytoplankton exhibit different, species-specific sensitivities to macrophyte allelochemicals (Gross, 2003; Hilt & Gross, 2008; Körner & Nicklisch, 2002; Mulderij, Mooij, Smolders, & van Donk, 2005; van Donk & van de Bund, 2002). Diatoms and cyanobacteria are often significantly inhibited by macrophyte allelochemicals, whereas chlorophytes appear less sensitive (Hilt & Gross, 2008). Different strains of the same species could also exhibit different sensitivities to macrophyte allelochemicals (Al-Shehri, 2010; Jasser, 1995; Körner & Nicklisch, 2002). Mulderij et al. (2005) reported a higher sensitivity of toxic than non-toxic *Microcystis aeruginosa* strains among cyanobacteria. Therefore, allelochemicals appear to be strong drivers of phytoplankton communities.

Although native macrophytes are an essential component of many freshwater communities, non-native invasive macrophytes can strongly impact the structure and functioning of the ecosystem (Fleming & Dibble, 2015; Vilà et al., 2010). Many exotic plants possess secondary compounds that are novel to the native community (Callaway & Ridenour, 2004; Macel, Vos, Jansen, Putten, & Dam, 2014). These novel compounds are known to be extremely bioactive (Cappuccino & Arnason, 2006), inhibiting the growth of both native plants and phytoplankton competitors (Hilt & Gross, 2008) and, thus, improving the fitness of invasive species and providing them with a strong competitive advantage over native species (Callaway & Ridenour, 2004; Kim & Lee, 2011). A better understanding of the phytoplankton sensitivity to invasive macrophyte allelochemicals would help explain the patterns of phytoplankton succession in invaded areas.

Invasive macrophyte species are capable of clonal reproduction by vegetative propagation and sexual reproduction by seed (Okada, Grewell, & Jasieniuk, 2009; Ren & Zhang, 2007). Low genetic diversity, resulting from the dispersal of vegetative propagules and clonal reproduction in introduced ranges, is disadvantageous for the long-term evolutionary potential of invasive populations (Barrett & Richardson, 1986). The water primrose *Ludwigia hexapetala* (syn. *L. grandiflora* subsp. *hexapetala*) is a South American species widely spread in western Europe and in the USA (Thouvenot, Haury, & Thiebaut, 2013). Dandelot, Robles, Pech, Cazaubon, and Verlaque (2008) showed that *L. hexapetala* inhibited the germination rate of the European native *Nasturtium officinale*, highlighting the allelopathic potential of this non-native invasive species. The level of environmental damage caused by the presence of dense stands of *L. hexapetala* is considerable: alteration of water flow, increase in sedimentation and accumulation of organic matter, induction of anoxic water conditions, an impairment of native plant, macroinvertebrate and fish populations (Dandelot, Matheron, Le Petit, Verlaque, & Cazaubon, 2005; Stiers, Crohain, Josens, & Triest, 2011). Recent studies in France have shown increasing numbers of *L. hexapetala* fertile populations over the past 15 years (Gillard, Grewell, Deleu, & Thiébaut, 2017; Haury et al., 2014; Ruaux, Greulich, Haury, & Berton, 2009), raising concerns about their invasiveness. Fruits of *L. hexapetala* float in water and are easily dispersed by water currents, as are the clonal (vegetative) propagules (Thouvenot et al., 2013). In this case, stimulation of its own seed germination or, conversely, inhibition of *L. hexapetala* seed germination by the native species mediated by allelochemicals could potentially exert a strong control on the persistence of *L. hexapetala* populations after disturbances such as managed control efforts (Haury et al., 2014). Indeed, while the majority of studies reports an inhibitory effect of allelochemicals, the allelopathic interactions also include the beneficial influences that a plant could exert over other plants or microorganisms through the release of allelochemicals (Chou, 1999; Fernandez et al., 2013; Rice, 1984).

As recently demonstrated by Grutters et al. (2017), invasive eudicot plant species with an emergent growth strategy, such as *L. hexapetala*, are most likely to possess strong allelopathic potential. In fact, emergent plant species generally contain higher levels of phenolic compounds and have been reported to exhibit double the allelopathic potential of submerged plant species (Grutters et al., 2017; Smolders, Vergeer, van der Velde, & Roelofs, 2000), thus necessitating a deeper understanding of their impact in ecosystem functioning in invaded areas.

Our main goals were to experimentally evaluate (i) whether the invasive macrophyte *L. hexapetala* can affect phytoplankton (chlorophyte *vs*. cyanobacteria and distinct strains within cyanobacteria); (ii)

whether both the *L. hexapetala* and a native species (*Mentha aquatica*) can affect the seed germination of *L. hexapetala*, as these two species co-occurred in the riverbanks; (iii) whether the plant's phenological stage could modulate these interactions; and (iv) what the allelochemicals involved in these interactions could be. On the basis of previous studies (Hilt & Gross, 2008; Mulderij et al., 2005), we hypothesised that H1: cyanobacteria would be more sensitive to *L. hexapetala* allelochemicals than chlorophytes and H2: that toxic cyanobacteria strains would be more sensitive than non-toxic ones. We also hypothesised H3: that *L. hexapetala* would promote its own seed germination and, on the contrary, H4: *M. aquatica* would inhibit seed germination of *L. hexapetala*. As the quantities and identities of the allelochemicals released into the environment vary according to the life stages of a given plant species (Hashoum et al., 2017; Lombardo, Mjelde, Källqvist, & Brettum, 2013), we hypothesised that H5: different plant phenological stages could exert distinct allelopathic effects.

2 | METHODS

2.1 | Plant and phytoplankton material used

Two axenic cyanobacterial strains, a toxic *M. aeruginosa* strain (PCC 7806), which produces microcystins and a non-producing *M. aeruginosa* strain (PCC 9432) obtained from the Pasteur Institute, and one chlorophyte species (unialgal culture of *Scenedesmus communis*, isolated in our laboratory) were selected as the target phytoplankton species. The three strains were mass-cultured in a BG-11 medium (2% of concentrated Sigma-Aldrich Cyanobacteria BG-11 Freshwater Solution 50× in deionised water) for 1 month prior to the experiment.

A hundred ripe seed capsules of *L. hexapetala* were collected during late summer in 2015 from the Apigné pond in Brittany, western France (48°05′37.9″N, 1°44′30.9″W). A maximum of three fruits were collected per erect stem, and samples were collected from individuals at least 10 m apart. Capsules were air-dried and stored for 6 months in the dark at 4°C prior to the experiment.

We collected leaves of *L. hexapetala* and *M. aquatica* in the spring, summer and autumn of 2016 in the Apigné pond. *L. hexapetala* exhibits two growth forms: a horizontal growth stage over water with small round leaves and a growth stage with erected elongated leaves. We collected the small round floating leaves (i.e. those in contact with water) on each of the sampling dates. The leaves were detached from the stems of several individuals, washed to remove benthic invertebrates and filamentous algae, and stored for 48 hr in the dark at 4°C prior to the experiment with leaf aqueous extracts.

2.2 | Allelopathic effects of leaf aqueous extracts

We chose to test the effects of natural leachates using leaf aqueous extracts because water-soluble compounds have been shown to be most involved in allelopathy (Fernandez et al., 2013; Gross, 2003).

Prior to the preparation of leaf aqueous extracts, five leaf samples, each weighing 10 g, were dried at 65°C for 48 hr to determine their dry weight and estimate the leaf water content according to the formula: (Fresh weight − Dry weight)/Fresh weight × 100%. The leaf water content of *L. hexapetala* and *M. aquatica* leaves was 90.5 (±0.3) % and 86.0 (±0.6) %, respectively. Leaf aqueous extracts of *L. hexapetala* and *M. aquatica* were prepared by soaking fresh leaves equivalent to 10 g of dry mass in 1,000 ml of deionised water for 12 hr in darkness. These 1% aqueous extracts were then filtered through a filter paper (Whatman #1). A part of the stock solution was diluted to 0.25% and the leaf aqueous extracts were then stored at 4°C for few hours prior to the experiment. We chose to test two low concentrations of leaf aqueous extracts (i.e. 1% and 0.25%) to mimic potential concentrations of natural leachates.

2.2.1 | Experiment no. 1: Allelopathic effects of *Ludwigia hexapetala* aqueous extracts on phytoplankton photosynthetic activity

The three phytoplankton strains (non-toxic *M. aeruginosa*, toxic *M. aeruginosa* and *S. communis*) were harvested by centrifugation (416 g for 20 min in 50 ml sterile centrifuge tubes) and re-suspended in 50 ml of a *L. hexapetala* leaf aqueous extract (1%, 0.25% or control, using deionised water as a diluent). Before this resuspension step, extract dilutions were filtered (using sterile 0.2 μm pore size membrane filter), enriched with 2% BG11 50× Sigma-Aldrich concentrated solution (to increase nutrient abundance) and pH adjusted to 7.1. Then, 50 ml of each phytoplankton suspension was poured onto five Petri dishes (50 mm diameter) and then randomly distributed in a climate-controlled room (Percival AR-41L3). The experiments were conducted in five replicates with extracts obtained from *L. hexapeala* in spring, summer and autumn 2016 (three phytoplankton strains × two extract concentrations plus control × three seasons × five replicates). Cultures were grown at 20°C, 60 μmol m^{-2} s^{-1} PAR and a 14 hr:10 hr light:dark photoperiod over 5 days.

To assess the allelopathic effect, we used pulse amplitude modulation (PAM) chlorophyll fluorescence yield to measure photosynthetic activity (Le Rouzic, Thiébaut, & Brient, 2016; Zhou et al., 2013). The fluorescence yield was measured after a 30 min dark adaptation with a Diving-PAM underwater fluorometer (Walz) after 6 hr, and then every 24 hr for 5 days. The initial fluorescence, F_0, and maximal fluorescence, F_m, were recorded using the weak measuring light and F_m after the saturation flash. The optimum quantum efficiency of PSII was calculated as $F_v/F_m = (F_m - F_o)/F_m$. The photosynthetic activity for each replicate was calculated as the average of the five time points during the 5-day experiment (CV = 4.3% to 17.9%).

2.2.2 | Experiment no. 2: Allelopathic effects of *Ludwigia hexapetala* and *Mentha aquatica* aqueous extracts on *Ludwigia hexapetala* seed germination ability

Thirty capsules of *L. hexapetala* were dissected for each season and a maximum of 25 seeds per capsule were retained for the experiment. Seed external surface was sterilised by immersion in a sodium

hypochlorite solution (5%) for 1 min and gently washed with distilled water. The bottom of sterilised Petri dishes (90 mm diameter) was covered with a layer of glass beads, a filter paper disc and 15 ml of distilled water. Twenty seeds were randomly distributed at regular intervals per Petri dish and stored at 4°C in darkness for 15 days to break seed dormancy. Thereafter, seeds present in the Petri dishes were watered with 15 ml of deionised water (control) or with 15 ml of a leaf aqueous extract (0.25% or 1%) of either *L. hexapetala* or *M. aquatica* and then randomly distributed in a climate-controlled room (Percival AR-41L3). The experiments were conducted in five replicates with extracts obtained from *L. hexapeala* and *M. aquatica* in spring, summer and autumn 2016 (two source species × two extract concentrations plus control × three seasons × five replicates). The bioassays were conducted under controlled conditions with a 12 hr:12 hr light:dark photoperiod and a 20°C:12°C light:dark temperature over 5 weeks. Seed germination was monitored three times a week.

A seed was considered to have germinated once the radicle protruded from the medium, after which it was removed from the Petri dish. The water level was maintained throughout the duration of the experiment by adding distilled water. The final germination percentage was calculated as (number of germinated seeds / the number of sown seeds) × 100. Germination velocity was calculated using the Kotowski velocity coefficient (Mazliak, 1982): $Cv = 100 \times (\Sigma Ni/\Sigma NiTi)$, where Ni is number of seeds germinated at time i, and Ti the number of days since the start of the experiment.

2.3 | Chemical analyses of leaves and leaf aqueous extracts

Samples of leaves (collected in the field) and leaf aqueous extracts (prepared for experiments no. 1 and no. 2) were freeze-dried and ground into powder prior to chemical analysis (Hashoum et al., 2017). The leaves and leaf aqueous extracts of *L. hexapetala* and *M. aquatica* were first analysed using liquid chromatography coupled to mass spectrometry (LCMS). Ten mg of the powder was mixed with 1 ml of methanol acidified with 1% formic acid and ultra-sonicated in a bath for 5 min. The sample was centrifuged (2000 g for 5 min) and 900 μl of the supernatant was filtered using a PTFE 13 mm 0.45 μm filter. The methanol extract was stored in a glass vial at 4°C until 2 μl was injected onto the chromatographic column for flavonol quantification. The reversed phase column (Waters Acquity C18 BEH, 2.1 × 150 mm, 1.7 μm) was maintained at 30°C. The solvents used for the binary gradient were as follows: A: ultrapure water with 0.1% formic acid and B: acetonitrile with 0.1% formic acid. The flow rate was 0.4 ml/min. The 25 min gradient applied was 97% A to 91% in 3 min, then 84% A in 5 min, 68% A in 7 min, 10% A in 3 min, a 3 min isocratic step, 2 min to 97% A and a 2 min isocratic step to re-equilibrate the column prior to a new sample. Flavonols were detected at 350 nm, by photo diode array. External quantification was conducted with mono glycosylated flavonols and hydroxycinnamic acid standards. The identity or structure of flavonols was confirmed with the triple quadrupole mass detector in full scan negative mode or targeted fragmentation. The capillary voltage was 2.9 kV, the cone voltage was 35 V, the source temperature was maintained at 150°C, the desolvation temperature was 400°C, and the desolvation gas flow was 800 L/h.

The total phenolic content (TPC) of leaves was also measured colorimetrically according to Santonja, Fernandez, Gauquelin, and Baldy (2015) using gallic acid for quantification. The leaf powder sample (250 mg) was suspended in 20 ml of a 70% aqueous methanol, shaken for 1 hr and then filtered (0.45 μm filter). The filtered extract (0.25 ml) was mixed with 4 ml of distilled water, 0.5 ml of saturated aqueous Na_2CO_3 and 0.25 ml of Folin–Ciocalteu reagent. After 60 min, TPC was determined at 765 nm on an UV/Vis spectrophotometer (Biomate 3; Thermo Electron Corporation®) and expressed as mg of gallic acid equivalent/g DW.

2.4 | Allelopathic effects of pure compounds

To retrieve the compounds involved in the allelopathic effects, we tested the effects of the three main compounds identified in the leaf aqueous extracts of *L. hexapetala*: myricitrin (myricetin 3-O-rhamnoside), prunin (naringenin-7-O-glucoside) and quercitrin (quercetin-3-O-rhamnoside) (Table 1) as pure compounds. The three tested compounds were glycosides. Myricitrin and quercitrin were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France) and prunin from Extrasynthese (Genay, France).

Concentrations tested were according to the content obtained in the leaf aqueous extract of the summer of 2016 (Table 1). These concentrations were highest in the *L. hexapetala* extract. The tested aqueous extracts (stock solution) were prepared by soaking 460 μg of myricitrin, or 160 μg of prunin, or 160 μg of quercitrin in 1,000 ml of deionised water. The solutions were then filtered through a filter paper (Whatman #1) and a part was diluted at 25% from stock solution (corresponding to concentrations of 115 μg of myricitrin, or 40 μg of prunin, or 40 μg of quercitrin/l deionised water), stored at 4°C.

2.4.1 | Experiment no. 3: Allelopathic effects of pure compounds on phytoplankton photosynthetic activity

The experiment was performed following the same procedure used in Experiment no. 1. Solutions of each pure compound (myricitrin or prunin or quercitrin) or deionised water for control were applied in five replicates per treatment. Cultures were grown at 20°C, 60 μmol m^{-2} s^{-1} PAR, and a 14 hr:10 hr light:dark photoperiod over 5 days.

2.4.2 | Experiment no. 4: Allelopathic effects of pure compounds on *Ludwigia hexapetala* seed germination

The experiment was performed following the same procedure used in Experiment no. 2. At the start of the experiment, each Petri dish

TABLE 1 Mean concentrations (±*SE*; *n* = 3) of the major chemical compounds found in the leaf aqueous extracts of *Ludwigia hexapetala* and *Mentha aquatica* in spring, summer and autumn. Leaf aqueous extracts were prepared by soaking 10 g of leaves in 1,000 ml of deionised water for 12 hr. Mean concentrations are expressed as μg/L. One-way ANOVAs were performed for differences among seasons for each chemical compound and for each plant separately. *F*-values and associated *p*-values are indicated. Different letters within a row denote significant differences between the three seasons with a < b < c (post hoc Tukey tests results after One-way ANOVA). *df* = Degree of freedom

	Spring	Summer	Autumn	*df*	*F*-value	*p*-value
Ludwigia hexapetala						
Myricitrin	88.0 (9.1) a	452.7 (3.8) c	334.4 (9.2) b	2	569.77	<0.0001
Prunin	56.6 (0.6) a	128.3 (0.8) b	121.5 (3.4) b	2	362.03	<0.0001
Quercitrin	97.7 (1.7) a	132.1 (1.0) b	141.9 (4.1) b	2	76.18	<0.0001
Mentha aquatica						
Caffeic acid	4.6 (0.7) a	14.4 (0.7) c	10.7 (0.6) b	2	56.12	0.0001
Chlorogenic acid	3.3 (0.2) a	6.0 (0.1) c	4.7 (0.1) b	2	124.76	<0.0001
p-Coumaric acid	4.3 (0.2) a	8.9 (0.5) b	4.1 (0.1) a	2	67.52	<0.0001
Rosmarinic acid	302.2 (39.8) a	942.1 (16.6) b	1,656.3 (25.8) c	2	547.57	<0.0001
Eriocitrin	91.6 (4.0) a	186.5 (3.6) b	272.6 (5.7) c	2	399.26	<0.0001
Eriodictyol	2.3 (0.1) a	4.3 (0.2) c	3.4 (0.2) b	2	43.58	0.0003

of the five replicates per treatment was watered with either 15 ml of deionised water or with an aqueous extract of each pure compound (myricitrin or prunin or quercitrin) and then randomly distributed in a climate-controlled room (Percival AR-41L3). The bioassays were conducted under controlled conditions with a 12 hr:12 hr light:dark photoperiod and a 20°C:12°C light:dark temperature over 5 weeks.

2.5 | Statistical analyses

Statistical analyses were performed with the R software (version 3.3.3). Significance was assumed at $p < 0.05$. The normality and homoscedasticity of the data showed that the data distributions met the requirements for use of parametric statistical analyses (using the Ryan-Joiner and Levene tests, respectively).

For experiment no. 1, the two-way ANOVAs, followed by Tukey HSD (honest significant difference) test for post hoc pairwise comparisons, were used to test concentration dependent effects (0%, 0.25% and 1.0%) and the season (spring, summer and autumn) on the photosynthetic activity of each phytoplankton strains separately (non-toxic *M. aeruginosa*, toxic *M. aeruginosa* and *S. communis*). For experiment no. 2, three-way ANOVAs, followed by Tukey HSD test for post hoc pairwise comparisons, were used to test for the effects of source species (*L. hexapetala* and *M. aquatica*), extract concentration (0%, 0.25% and 1%) and season (spring, summer and autumn) on germination rate and velocity of *L. hexapetala* seeds. For experiments no. 3 and no. 4, one-way ANOVAs, followed by Tukey HSD test for post hoc pairwise comparisons, were used to test for effects of the three pure compounds (myricitrin, prunin and quercitrin) on photosynthetic activity of the three phytoplankton strains (non-toxic *M. aeruginosa*, toxic *M. aeruginosa* and *S. communis*) and on germination rate and velocity of *L. hexapetala* seeds.

3 | RESULTS

3.1 | Allelopathic effects of leaf aqueous extracts on phytoplankton photosynthetic activity

The photosynthetic activity of *S. communis* was higher in autumn than in spring or summer (Table 2). The photosynthetic activity of the toxic *M. aeruginosa* decreased from spring to summer to autumn (Table 2), whereas the photosynthetic activity of the non-toxic *M. aeruginosa* was not affected by the season (Table 2). As indicated by the significant interaction of extract concentration × season (Table 2), the allelopathic effects on phytoplankton photosynthetic activity strongly depended on the season. Indeed, the photosynthetic activity of *S. communis* increased in the presence of leaf aqueous extract only in autumn (Figure 1a), the photosynthetic activity of the non-toxic *M. aeruginosa* decreased according to the increase in extract concentration in spring and autumn (Figure 1b), and the photosynthetic activity of toxic *M. aeruginosa* increased in the presence of leaf aqueous extract only in spring (Figure 1c). Finally, the three phytoplankton strains showed a similar trend in summer with no effect when the extract concentration was low and a strong decrease in their photosynthetic activities when the extract concentration was high (Figure 1).

3.2 | Allelopathic effects of leaf aqueous extracts on *Ludwigia hexapetala* seed germination

The germination rate was strongly affected by the season, with a higher germination rate in summer than in spring and autumn (Table 3). The germination rate of *L. hexapetala* increased with extract concentration and was similarly affected by *M. aquatica* and *L. hexapetala* leaf aqueous extracts (Table 3). However, the

TABLE 2 Analysis of variance to test for the effects of extract concentration (0 %, 0.25 % and 1.0 %) and season (spring, summer and autumn) on the photosynthetic activity of the three phytoplankton strains (*Scenedesmus communis*, non-toxic *Microcystis aeruginosa* and toxic *Microcystis aeruginosa*). *df* = degrees of freedom, % SS = percentage sums of squares. *F*-values and associated *p*-values are indicated

	S. communis				Non-toxic *M. aeruginosa*				Toxic *M. aeruginosa*			
	df	%SS	*F*-value	*p*-value	*df*	%SS	*F*-value	*p*-value	*df*	%SS	*F*-value	*p*-value
Concentration	2	8.95	4.44	0.0188	2	83.81	126.92	<0.0001	2	5.87	11.76	0.0001
Season	2	16.42	8.15	0.0012	2	0.09	0.13	0.8785	2	66.08	132.39	<0.0001
Concentration × season	4	38.36	9.52	<0.0001	4	4.22	3.19	0.0241	4	19.06	19.09	<0.0001
Residuals	36	36.26			36	11.89			36	8.98		

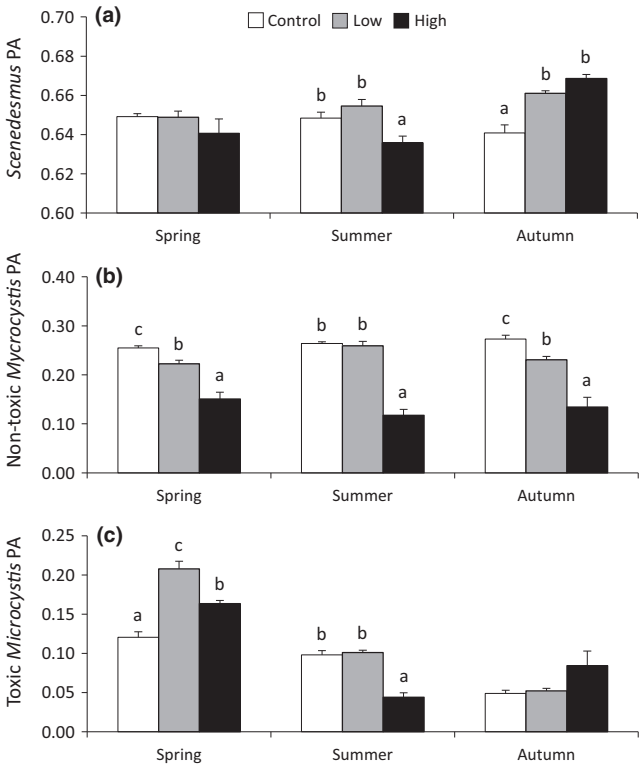

FIGURE 1 Mean values (±*SE*; *n* = 5) of photosynthetic activity PA of *Scenedesmus communis* (a), the non-toxic *Microcystis aeruginosa* (b) and the toxic *Microcystis aeruginosa* (c) according to the interaction of extract concentration × season. Mean PA values are expressed as optimum quantum efficiencies of PSII (F_v/F_m). The *y*-axis scales vary among panels. Different letters denote significant differences between treatments with a < b < c (post hoc Tukey tests)

allelopathic effects on the germination rate varied strongly depending on the season (interaction of extract concentration × season, Table 3). Indeed, the germination rate increased at high concentration of extracts in spring, while it increased at low concentration in autumn (Figure 2a).

Similar to the germination rate, the germination velocity was strongly affected by the season with an increase in germination velocity according to the gradient from spring to summer to autumn (Table 3). The germination velocity increased according to the extract concentration (Table 3) and was similarly affected by *M. aquatica* and *L. hexapetala* leaf aqueous extracts (Table 3). However, the extent to which the extract concentration affected the germination velocity varied according to the season (interaction of extract concentration × season, Table 3). It was not affected in spring, increased in summer, and increased only when the extract concentration was high in autumn (Figure 2b).

3.3 | Chemical analyses of leaves and leaf aqueous extracts

The UPLC-PDA-ESI-TQD analysis of *L. hexapetala* extracts revealed the presence of three flavonoid glycosides (myricitrin, prunin and quercitrin) as major compounds of the leaves (Supporting Information Table S1) and in the leaf aqueous extracts (Table 1). The UPLC-PDA-ESI-TQD analysis of *M. aquatica* extracts revealed the presence of two simple phenols (caffeic acid and p-coumaric acid), two simple glycosylated phenols (chlorogenic acid and rosmarinic acid) and two flavonoids (eriocitrin and eriodictyol) as the major compounds of the leaves (Supporting Information Table S1) and the leaf aqueous extracts (Table 1).

Compound concentrations in leaf aqueous extracts showed a clear seasonal pattern with the lowest concentrations being found in spring (Table 1) and the highest concentrations being found in summer for several compounds (myricitrin, caffeic acid, chlorogenic acid, p-coumaric acid, eriodictyol; Table 1). For two compounds found in the *L. hexapetala* leaf aqueous extracts (prunin and quercitrin), we observed the same level of concentration in the summer and the autumn (Table 1). For two compounds found in the *M. aquatica* leaf aqueous extracts (rosmarinic acid and eriocitrin), we observed an increase in concentration according to the leaf's phenological stage (i.e. increasing from spring to summer to autumn; Table 1).

The total phenolic content (TPC) was two times higher in the *L. hexapetala* than in the *M. aquatica* leaves ($F_{1,18}$ = 878.77, p < 0.0001), at 135.5 ± 5.0 and 53.5 ± 1.9 mg/g DW, respectively. We also found an increase in TPC according to the phenological stage in both species ($F_{2,18}$ = 20.42, p = 0.0001), with higher TPC in summer and autumn (144.2 ± 3.6 and 56.9 ± 1.3 mg/g DW for *L. hexapetala* and *M. aquatica*, respectively) than in spring (118.3 ± 4.0 and 46.8 ± 1.0 mg/g DW for *L. hexapetala* and *M. aquatica*, respectively).

TABLE 3 Analysis of variance to test for the effects of plant source species (*Ludwigia hexapetala* and *Mentha aquatica*), extract concentration (0 %, 0.25 % and 1 %) and season (spring, summer and autumn) on germination rate and germination velocity of *L. hexapetala* seeds. *df* = degrees of freedom, % SS = percentage sums of squares. *F*-values and associated *p*-values are indicated

	Germination rate				Germination velocity			
	df	%SS	*F*-value	*p*-value	*df*	%SS	*F*-value	*p*-value
Species	1	0.02	0.10	0.7626	1	0.03	0.06	0.8074
Concentration	2	6.77	12.49	<0.0001	2	8.51	9.29	0.0003
Season	2	57.56	106.13	<0.0001	2	47.52	51.93	<0.0001
Species × concentration	2	1.27	2.34	0.1034	2	0.27	0.29	0.7481
Species × season	2	2.05	3.78	0.0276	2	0.46	0.50	0.6101
Concentration × season	4	9.31	8.59	<0.0001	4	12.54	6.85	0.0001
Species × concentration × season	4	3.49	3.21	0.0175	4	1.39	0.76	0.5546
Residuals	72	19.53			64	29.28		

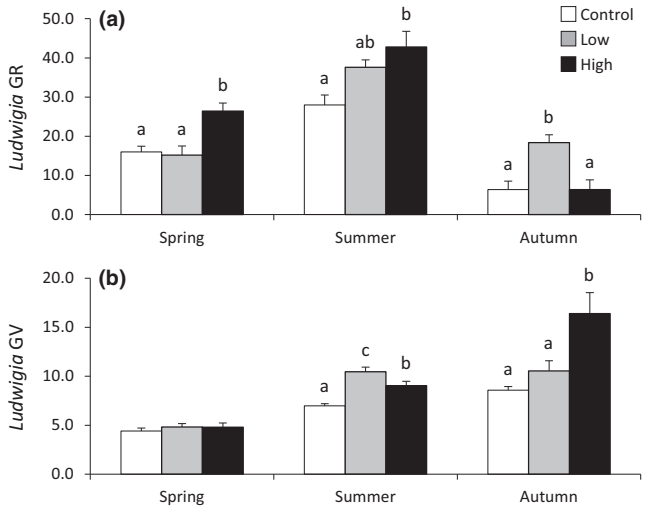

FIGURE 2 Mean values (±*SE*; *n* = 5) of germination rate GR (a) and germination velocity GV (b) of *Ludwigia hexapetala* seeds according the interaction of extract concentration × season. Mean GR values are expressed as percentage of seeds that germinated. Different letters denote significant differences between treatments with a < b < c (post hoc Tukey tests)

3.4 | Allelopathic effects of pure compounds

The photosynthetic activity of *S. communis* was higher in the presence of prunin, quercitrin and, to a lesser extent, myricitrin than in allelochemical-free controls (Table 4; Figure 3a). The photosynthetic activity of the non-toxic *M. aeruginosa* was lower in the presence of prunin compared with the control treatment (Table 4; Figure 3b). Contrastingly, the photosynthetic activity of the toxic *M. aeruginosa* was higher in the presence of myricitrin and, to a lesser extent, quercitrin than in allelochemical-free controls (Table 4; Figure 3c).

The germination rate and the germination velocity of *L. hexapetala* seeds were on average two times higher in the presence of myricitrin when compared to the control treatment (Table 4; Figures 4a, b). These two germination parameters were also on average two times higher in the presence of prunin only when the compound concentration was low (Table 4; Figures 4a, b) and they were not affected by the presence of quercitrin (Table 4; Figures 4a, b).

4 | DISCUSSION

4.1 | Allelopathic effects of leaf aqueous extracts on phytoplankton

The allelopathic activity of *L. hexapetala* towards phytoplankton was demonstrated by both negative and positive effects of leaf aqueous extracts on phytoplankton photosynthetic activity (as a proxy of phytoplankton primary production). *Ludwigia hexapetala* strongly repressed the non-toxic *M. aeruginosa* strain photosynthetic activity and, on the contrary, stimulated toxic *M. aeruginosa* in spring and *S. communis* strains in autumn. These differential effects are in line with the general consensus that allelopathic effects can be highly species-specific (Gross, Hilt, Lombardo, & Mulderij, 2007; Körner & Nicklisch, 2002; Le Rouzic et al., 2016; van Donk & van de Bund, 2002). The allelopathic effect on *S. communis* was low with only 2% stimulation, in agreement with previous studies that also reported *Scenedesmus* as relatively insensitive to macrophyte allelochemicals (Hilt & Gross, 2008; Jasser, 1995; Körner & Nicklisch, 2002; Lürling, van Geest, & Scheffer, 2006). Oxidative damage has been considered as one of the important allelopathic and toxicological mechanisms of allelochemicals (Gniazdowska & Bogatek, 2005; Mohamed, 2017). A differential physiological response between chlorophyte and cyanobacteria against such induced oxidative stress may explain their relative survival capacity (Le Rouzic et al., 2016; Zhang, Wang, He, & Zhang, 2011).

Our results demonstrated different effects between the two strains of *M. aeruginosa*. Non-toxic *M. aeruginosa* was strongly inhibited (49%), in agreement with a recent study highlighting the strong inhibitory effect of *L. hexapetala* aqueous extracts on the cyanobacterium *Dolichospermum flos-aquae* (Grutters et al., 2017). Surprisingly, and in contrast with previous studies (Mulderij et al., 2005; Takeda

TABLE 4 Analysis of variance to test for the effects of the three pure compounds (myricitrin, prunin and quercitrin) on the photosynthetic activity of the three phytoplankton strains (*Scenedesmus communis*, non-toxic *Microcystis aeruginosa* and toxic *Microcystis aeruginosa*) and on the germination of *Ludwigia hexapetala* seeds (germination rate and velocity). *F*-values and associated *p*-values are indicated

	S. communis		NT *M. aeruginosa*		Toxic *M. aeruginosa*		Germination rate		Germination velocity	
	F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Myricitrin	5.16	0.0241	0.07	0.9368	11.29	0.0017	17.71	0.0003	21.53	0.0001
Prunin	14.06	0.0007	18.88	0.0002	1.17	0.3424	6.63	0.0115	11.91	0.0014
Quercitrin	4.85	0.0286	1.25	0.3242	6.94	0.0100	0.07	0.9290	1.73	0.2182

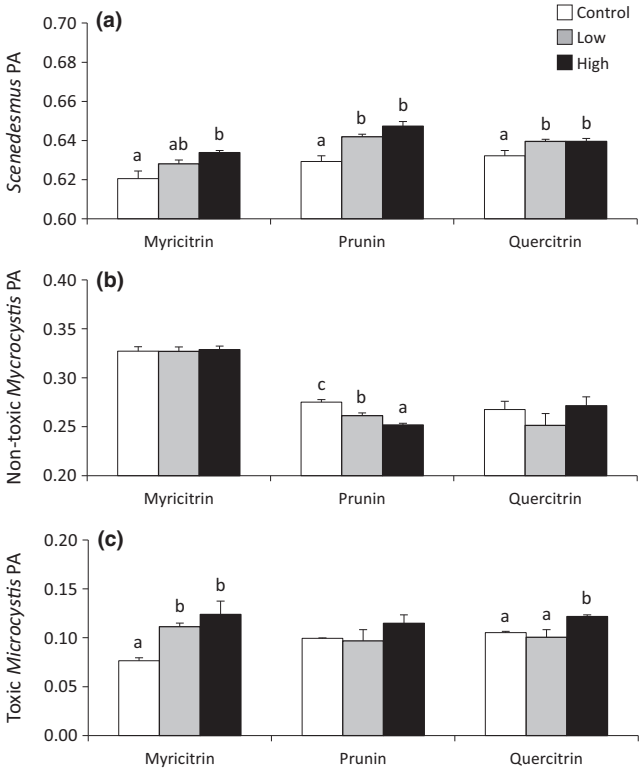

FIGURE 3 Mean values (±*SE*; $n = 5$) of photosynthetic activity PA of *Scenedesmus communis* (a), non-toxic *Microcystis aeruginosa* (b) and toxic *Microcystis aeruginosa* (c) treated with three pure compounds (myricitrin, prunin and quercitrin). Mean PA values are expressed as optimum quantum efficiencies of PSII (F_v/F_m). The *y*-axis scales vary among panels. Different letters denote significant differences between treatments with a < b < c (post hoc Tukey tests)

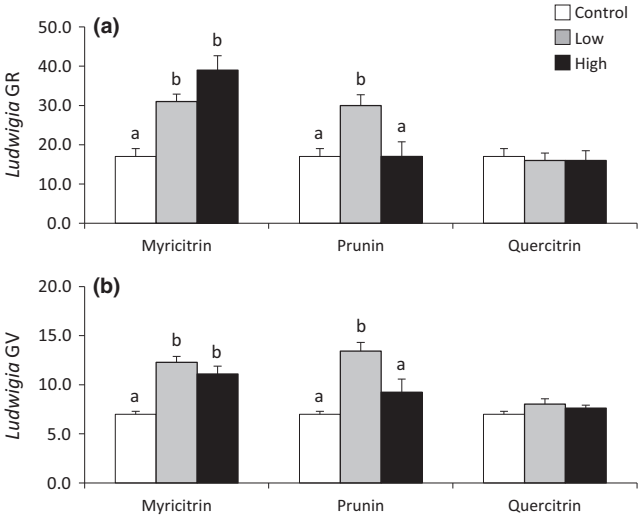

FIGURE 4 Mean values (±*SE*) of germination rate GR (a) and germination velocity GV (b) of *Ludwigia hexapetala* seeds treated with three pure compounds (myricitrin, prunin and quercitrin). Mean GR values are expressed as percentage of seeds that germinated. Different letters denote significant differences between treatments with a < b < c (post hoc Tukey tests)

et al., 2011), the toxic *M. aeruginosa* was strongly stimulated (35%) leading to a totally opposite response between these two *Microcystis* strains in spring. These differences in sensitivity at intraspecific levels could be attributed to differences in the attached microbial biofilm, energy allocation, cell wall properties, uptake mechanisms or sensitivity to induced oxidative stress (Casamatta & Wickstrom, 2000; Mulderij et al., 2005).

However, we observed a strong seasonal effect of allelopathic interactions. As expected, the allelochemical composition of the leaf aqueous extracts varied according to the life stages of *L. hexapetala* and thus exhibited distinct allelopathic effects. Previous studies have also reported a seasonal pattern of allelopathic interactions (Bauer et al., 2009; Hilt, Ghobrial, & Gross, 2006; Lombardo et al., 2013). Bauer et al. (2009) reported a maximum inhibitory effect of *Myriophyllum verticillatum* on the cyanobacterium *Anabaena variabilis* in May and June (i.e. spring), while Lombardo et al. (2013) reported an inhibitory effect of *Ceratophyllum demersum* on the cyanobacterium *Synechococcus leopoliensis* from June to August but not in September. The seasonal dependence of biotic interactions has been poorly studied, and here, we point to the necessity to take this into account to better understand macrophyte–phytoplankton interactions. In addition to the shifts in released allelochemicals according to the plant life stages highlighted in this study, other factors such as plant biomass, plant growth, resources (e.g. light or nutrients), temperature and hydrodynamics across seasons could also influence the outcome of biotic interactions in the field (Gross, 2003; Hilt et al., 2006; Lombardo et al., 2013). Here, we provide evidence that the presence of *L. hexapetala* would be expected to result in phytoplankton communities with higher dominance of toxic cyanobacteria than non-toxic ones in spring and autumn. Moreover, by stimulating toxic cyanobacteria in spring and inhibiting this strain in summer, *L. hexapetala* can

respectively favour and reduce toxic cyanobacteria bloom leading to drastic changes in the functioning of invaded ponds.

4.2 | Allelopathic effects of leaf aqueous extracts on seed germination

Seed germination is one of the main life stages usually affected by allelochemicals by inhibition of seed germination (Dandelot et al., 2008; Fernandez et al., 2013) or delaying of germination (Fernandez et al., 2013; Hashoum et al., 2017), impairing nutrient uptake, membrane permeability, or cell division and morphology (Inderjit & Duke, 2003; Rice, 1984).

Previous experiments focused on interspecific plant–plant interactions and tested the allelopathic effect of *Ludwigia* spp. on germination and seedling growth of other target species (Dandelot et al., 2008; Sakpere, Oziegbe, & Bilesanmi, 2010). However, when allelochemicals are released into the environment, a plant species could also stimulate its own germination, as shown for *Pennisetum glaucum* and four *Chenopodiaceae* species (Jefferson & Pennacchio, 2003; Saxena, Singh, & Joshi, 1996). Here, we observed a strong stimulation of *L. hexapetala* seed germination by *L. hexapetala* leaf aqueous extracts, suggesting that *L. hexapetala* could promote their own population persistence. Contrary to the expectations, *L. hexapetala* seed germination also was stimulated by *M. aquatica* leaf aqueous extracts. This stimulation by *M. aquatica* is all the more surprising since this plant releases allelochemicals, such as gallic acid and p-coumaric acids, known to be strong inhibitors of seed germination (Reigosa & Pazos-Malvido, 2007). Indeed, these positive allelopathic interactions could give a strong competitive advantage to *L. hexapetala* with cascading consequences on the structure and composition of the plant community.

We also observed a strong seasonal effect of the seed germination stimulation, in accordance with previous studies. For example, Dandelot et al. (2008) showed that *L. hexapetala* inhibited the germination rate of *Nasturtium officinale* only in summer. In our experiments, we observed a higher seed germination stimulation in summer compared with spring and autumn. As warmer conditions are favourable for increasing germination rate (Gillard, Grewell, Futrell, Deleu, & Thiébaut, 2017), improving seed germination success during summer could give a strong competitive advantage to *L. hexapetala*.

4.3 | Allelopathic effects of pure compounds

Phenolic compounds are currently the compounds most consistently related to allelopathic interactions in freshwater ecosystems (Gross, 2003; Iason, Dicke, & Hartley, 2012; Leu, Krieger-Liszkay, Goussias, & Gross, 2002), including the induction of allelopathic effects by aquatic plants against phytoplankton (Bauer et al., 2009; Gross et al., 2007; Grutters et al., 2017) or between aquatic plant species (Dandelot et al., 2008; Elakovitch & Wooten, 1989). The total phenolic content (TPC) of *L. hexapetala* leaves observed in this study is very close to the TPC recently observed by Grutters et al. (2017) that identified *Ludwigia* species as macrophytes with a high allelopathic potential. The main secondary compounds released by *L. hexapetala* are three flavonoid glycosides belonging to the group of polyphenols that are known to play a substantial role in allelopathic interactions. For example, Huang et al. (2015) recently reported strong negative effects of three flavonoids (5, 4′-dihydroxyflavone, apigenin and luteolin) on the photosynthetic activity and the cell integrity of *M. aeruginosa*. Nakai, Inoue, Hosomi, and Murakami (2000) showed that ellagic, gallic and pyrogallic acids and catechin inhibited the growth of *M. aeruginosa*. In this study, for the first time, we reported the allelopathic effects of myricitrin, prunin and quercitrin against *M. aeruginosa*. Prunin inhibited the photosynthetic activity of a non-toxic *M. aeruginosa* strain. In contrast, myricitrin and quercitrin stimulated the photosynthetic activity of a toxic *M. aeruginosa* strain. This important finding suggests that aquatic plants releasing such phenolics would favour toxic *M. aeruginosa* strains at the expense of non-toxic ones. In line with our experiment with leaf aqueous extracts, the three flavonoids slightly stimulated the photosynthetic activity of *S. communis*, suggesting they could all be involved in this positive allelopathic effect. However, the individual effect of such three compounds cannot fully explain the results obtained in the experiment using the leaf aqueous extracts, especially the strong inhibiting effect observed in summer. As previously observed by Wu et al. (2009), phenolic compounds could exert synergetic effects on phytoplankton growth.

The seed germination of *L. hexapetala* was stimulated by the myricitrin and prunin that are released in its leaf aqueous extracts. Myricitrin and prunin, as well as their aglycones (i.e. myricetin and naringenin), have previously been reported as feeding deterrents against herbivore insects, toxic compounds against microorganisms and allelopathic compounds inhibiting plant growth (Deng, Aoki, & Yogo, 2004; Iwashina, 2003; Nicollier & Thompson, 1983). Surprisingly, for the first time, we report a seed germination stimulation by these two compounds. However, as their effect on the seed germination and the physiological processes involved are unknown, further studies are required to better understand this stimulatory effect.

Despite the fact that we did not directly test the compounds released by *M. aquatica*, we identified the main phenolics released in its leaf aqueous extracts. Compounds such as caffeic acid (Nakai, Inoue, & Hosomi, 2001; Wang et al., 2013) or p-coumaric acid (Nakai et al., 2001; Zhang, Zheng, Hu, Xu, & Wang, 2010) are known to be strong growth inhibitors of both toxic and non-toxic cyanobacteria. Release of such phenolics by the native *M. aquatica* could limit cyanobacteria growth and maintain a clear water state in a freshwater pond. As allelochemicals released by *L. hexapetala* could stimulate toxic cyanobacteria, the outcome of the competition between these two macrophytes would have major consequences for the functioning of freshwater ecosystems in the invaded areas.

To conclude, our study pointed out complex interactions between native and non-native invasive macrophytes and different phytoplankton species depending on macrophyte life stage. The seed germination of *L. hexapetala* was stimulated by both *L. hexapetala*

and *M. aquatica*, suggesting a strong competitive advantage in favour of the invasive macrophyte with potential cascading consequences on the structure and composition of the plant community. Here, we also provided clear evidence that the presence of *L. hexapetala* would be expected to result in phytoplankton communities with higher dominance of toxic cyanobacteria than non-toxic ones in spring and autumn. By stimulating toxic cyanobacteria in spring and inhibiting this strain in summer, *L. hexapetala* can respectively favour and reduce toxic cyanobacteria bloom leading to drastic changes in the functioning of invaded ponds. Regardless, our findings confirm prior studies that recommend the development of an effective management (possibly eradication) of *L. hexapetala* in invaded areas.

ACKNOWLEDGMENTS

We would especially like to thank Oriane Chesneau, Morgane Milin, Elissia Mourey, Anais Rancon and Marc Ravatin for their assistance during the material collection and laboratory experiments, and Nathalie Marnet for technical assistance during the chemical analysis. Chemical analysis was performed at "Plateau de Profilage Métabolique et Métabolomique" (P2M2, INRA, Le Rheu, France). We thank Elisabeth Gross for helpful discussion, as well as Claudia Wiegand, two anonymous reviewers and the associate editor Alan Steinman for thoughtful comments that improved a previous version of our manuscript. This study was funded by the Axe "Biological Invasions" (ECOBIO, Rennes, France).

AUTHORS' CONTRIBUTIONS

MS, BLR and GT conceived and performed the experiments. MS analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ORCID

Mathieu Santonja http://orcid.org/0000-0002-6322-6352

REFERENCES

Al-Shehri, A. M. (2010). Differential sensitivity of different *Scenedesmus obliquus* strains to the allelopathic activity of the macrophyte *Stratiotes aloides*. *Journal of Applied Science*, *10*, 1769–1774.

Barrett, S. C. H., & Richardson, B. J. (1986). Genetic attributes of invading species. In R. H. Groves, & J. J. Burdon (Eds.), *Ecology of Biological Invasions* (pp. 21–33). Melbourne, Vic.: Cambridge University Press.

Bauer, N., Blaschke, U., Beutler, E., Gross, E. M., Jenett-Siems, K., Siems, K., & Hilt, S. (2009). Seasonal and interannual dynamics of polyphenols in *Myriophyllum verticillatum* and their allelopathic activity on *Anabaena variabilis*. *Aquatic Botany*, *91*, 110–116. https://doi.org/10.1016/j.aquabot.2009.03.005

Callaway, R. M., & Ridenour, W. M. (2004). Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, *2*, 436–443. https://doi.org/10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2

Cappuccino, N., & Arnason, J. T. (2006). Novel chemistry of invasive exotic plants. *Biology Letters*, *2*, 189–193. https://doi.org/10.1098/rsbl.2005.0433

Casamatta, D. A., & Wickstrom, C. E. (2000). Sensitivity of two disjunct bacterioplankton communities to exudates from the cyanobacterium *Microcystis aeruginosa* Kützing. *Microbial Ecology*, *40*, 64–73. https://doi.org/10.1007/s002480000035

Chou, C. H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture. *Critical Reviews in Plant Sciences*, *18*, 609–636. https://doi.org/10.1080/07352689991309414

Dandelot, S., Matheron, R., Le Petit, J., Verlaque, W., & Cazaubon, A. (2005). Temporal variations of physicochemical and microbiological parameters in three freshwater ecosystems (southeastern France) invaded by *Ludwigia* spp. *Comptes Rendus Biologies*, *328*, 991–999. https://doi.org/10.1016/j.crvi.2005.09.007

Dandelot, S., Robles, C., Pech, N., Cazaubon, A., & Verlaque, R. (2008). Allelopathic potential of two invasive alien *Ludwigia* spp. *Aquatic Botany*, *88*, 311–316. https://doi.org/10.1016/j.aquabot.2007.12.004

Declerck, S. A., Bakker, E. S., van Lith, B., Kersbergen, A., & van Donk, E. (2011). Effects of nutrient additions and macrophyte composition on invertebrate community assembly and diversity in experimental ponds. *Basic and Applied Ecology*, *12*, 466–475. https://doi.org/10.1016/j.baae.2011.05.001

Deng, F., Aoki, M., & Yogo, Y. (2004). Effect of naringenin on the growth and lignin biosynthesis of gramineous plants. *Weed Biology and Management*, *4*, 49–55. https://doi.org/10.1111/j.1445-6664.2003.00119.x

Elakovitch, S. D., & Wooten, J. W. (1989). Allelopathic potential of sixteen aquatic and wetland plants. *Toxicology*, *17*, 129–182.

Fernandez, C., Santonja, M., Gros, R., Monnier, Y., Chomel, M., Baldy, V., & Bousquet-Melou, A. (2013). Allelochemicals of *Pinus Halepensis* as drivers of biodiversity in Mediterranean open mosaic habitats during the colonization stage of secondary succession. *Journal of Chemical Ecology*, *39*, 298–311. https://doi.org/10.1007/s10886-013-0239-6

Fleming, J. P., & Dibble, E. D. (2015). Ecological mechanisms of invasion success in aquatic macrophytes. *Hydrobiologia*, *746*, 23–37. https://doi.org/10.1007/s10750-014-2026-y

Gillard, M., Grewell, B. J., Deleu, C., & Thiébaut, G. (2017). Climate warming and water primroses: Germination responses of populations from two invaded ranges. *Aquatic Botany*, *136*, 155–163. https://doi.org/10.1016/j.aquabot.2016.10.001

Gillard, M., Grewell, B. J., Futrell, C. J., Deleu, C., & Thiébaut, G. (2017). Germination and seedling growth of water primroses: A cross experiment between two invaded ranges with contrasting climates. *Frontiers in Plant Science*, *8*, 1677. https://doi.org/10.3389/fpls.2017.01677

Gniazdowska, A., & Bogatek, R. (2005). Allelopathic interactions between plants. Multi site action of allelochemical. *Acta Physiologiae Plantarum*, *27*, 395–407. https://doi.org/10.1007/s11738-005-0017-3

Gross, E. M. (2003). Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences*, *22*, 313–339. https://doi.org/10.1080/713610859

Gross, E. M., Hilt, S., Lombardo, P., & Mulderij, G. (2007). Searching for allelopathic effects of submerged macrophytes on phytoplankton – state of the art and open questions. *Hydrobiologia*, *584*, 77–88. https://doi.org/10.1007/s10750-007-0591-z

Grutters, B., Saccomanno, B., Gross, E. M., van de Waal, D. B., van Donk, E., & Bakker, E. S. (2017). Growth strategy, phylogeny and stoichiometry determine the allelopathic potential of native and non-native plants. *Oikos*, *12*, 1770–1779. https://doi.org/10.1111/oik.03956

Hashoum, H., Santonja, M., Gauquelin, T., Saatkamp, A., Gavinet, A., Greff, S., … Bousquet-Mélou, A. (2017). Biotic interactions in a Mediterranean oak forest: Role of allelopathy along phenological development of woody species. *European Journal of Forest Research*, *136*, 699–710. https://doi.org/10.1007/s10342-017-1066-z

Haury, J., Druel, A., Cabral, T., Paulet, Y., Bozec, M., & Coudreuse, J. (2014). Which adaptations of some invasive *Ludwigia* spp. (Rosidae, Onagraceae) populations occur in contrasting hydrological conditions in Western France? *Hydrobiologia*, *737*, 45–56. https://doi.org/10.1007/s10750-014-1815-7

Hilt, S., Ghobrial, M. G. N., & Gross, E. M. (2006). In situ allelopathic potential of *Myriophyllum verticillatum* (Haloragaceae) against selected phytoplankton species. *Journal of Phycology*, *42*, 1189–1198. https://doi.org/10.1111/j.1529-8817.2006.00286.x

Hilt, S., & Gross, E. M. (2008). Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology*, *9*, 422–432. https://doi.org/10.1016/j.baae.2007.04.003

Huang, H., Xiao, X., Ghadouani, A., Wu, J., Nie, Z., Peng, C., … Shi, J. (2015). Effects of natural flavonoids on photosynthetic activity and cell integrity in *Microcystis aeruginosa*. *Toxins*, *7*, 66–80. https://doi.org/10.3390/toxins7010066

Iason, G. R., Dicke, M., & Hartley, S. E. (2012). *The ecology of plant secondary metabolites: From genes to global processes*. New York, NY: Cambridge University Press.

Inderjit, S., & Duke, S. O. (2003). Ecophysiological aspects of allelopathy. *Planta*, *217*, 529–539.

Iwashina, T. (2003). Flavonoid function and activity to plants and other organisms. *Biological Sciences in Space*, *17*, 24–44. https://doi.org/10.2187/bss.17.24

Jasser, I. (1995). The influence of macrophytes on a phytoplankton community in experimental condition. *Hydrobiologia*, *306*, 21–32. https://doi.org/10.1007/BF00007855

Jefferson, L. V., & Pennacchio, M. (2003). Allelopathic effects of foliage extracts from four *Chenopodiaceae* species on seed germination. *Journal of Arid Environments*, *55*, 275–285. https://doi.org/10.1016/S0140-1963(03)00028-4

Kim, Y. O., & Lee, E. J. (2011). Comparison of phenolic compounds and the effects of invasive and native species in East Asia: Support for the novel weapons hypothesis. *Ecological Research*, *26*, 87–94. https://doi.org/10.1007/s11284-010-0762-7

Körner, S., & Nicklisch, A. (2002). Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. *Journal of Phycology*, *38*, 862–871. https://doi.org/10.1046/j.1529-8817.2002.t01-1-02001.x

Le Rouzic, B., Thiébaut, G., & Brient, L. (2016). Selective growth inhibition of cyanobacteria species (*Planktothrix agardhii*) by a riparian tree leaf extract. *Ecological Engineering*, *97*, 74–78. https://doi.org/10.1016/j.ecoleng.2016.07.021

Leu, E., Krieger-Liszkay, A., Goussias, C., & Gross, E. M. (2002). Polyphenolic allelochemicals from the aquatic angiosperm *Myriophyllum spicatum* inhibit photosystem II. *Plant Physiology*, *130*, 2011–2018. https://doi.org/10.1104/pp.011593

Lombardo, P., Mjelde, M., Källqvist, T., & Brettum, P. (2013). Seasonal and scale-dependent variability in nutrient-and allelopathy-mediated macrophyte–phytoplankton interactions. *Knowledge and Management of Aquatic Ecosystems*, *409*, 10. https://doi.org/10.1051/kmae/2013055

Lürling, M., van Geest, G., & Scheffer, M. (2006). Importance of nutrient competition and allelopathic effects in suppression of the green alga *Scenedesmus obliquus* by the macrophytes *Chara*, *Elodea* and *Myriophyllum*. *Hydrobiologia*, *556*, 209–220. https://doi.org/10.1007/s10750-005-1168-3

Macel, M., Vos, R. C., Jansen, J. J., Putten, W. H., & Dam, N. M. (2014). Novel chemistry of invasive plants: Exotic species have more unique metabolomic profiles than native congeners. *Ecology and Evolution*, *4*, 2777–2786. https://doi.org/10.1002/ece3.1132

Mazliak, P. (1982). *Physiologie végétale: Croissance et développement*. Paris, France: Hermann Publication.

Mohamed, Z. A. (2017). Macrophytes-Cyanobacteria allelopathic interactions and their implications for water resources management – A review. *Limnologica*, *63*, 132–142.

Mulderij, G., Mau, B., van Donk, E., & Gross, E. M. (2007). Allelopathic activity of *Stratiotes aloides* on phytoplankton – towards identification of allelopathic substances. *Hydrobiologia*, *584*, 89–100. https://doi.org/10.1007/s10750-007-0602-0

Mulderij, G., Mooij, W. M., Smolders, A. J. P., & van Donk, E. (2005). Allelopathic inhibition of phytoplankton by exudates from *Stratiotes aloides*. *Aquatic Botany*, *82*, 284–296. https://doi.org/10.1016/j.aquabot.2005.04.001

Nakai, S., Inoue, Y., & Hosomi, M. (2001). Algal growth inhibition effects and inducement modes by plant-producing phenols. *Water Research*, *35*, 1855–1859. https://doi.org/10.1016/S0043-1354(00)00444-9

Nakai, S., Inoue, Y., Hosomi, M., & Murakami, A. (2000). *Myriophyllum spicatum* released allelopathic polyphenols inhibiting growth of blue-green algae *Microcystis aeruginosa*. *Water Research*, *34*, 3026–3032. https://doi.org/10.1016/S0043-1354(00)00039-7

Nicollier, G., & Thompson, A. C. (1983). Flavonoids of *Desmanthus illinoensis*. *Journal of Natural Products*, *46*, 112–117. https://doi.org/10.1021/np50025a011

Okada, M., Grewell, B. J., & Jasieniuk, M. (2009). Clonal spread of invasive *Ludwigia hexapetala* and *L. grandiflora* in freshwater wetlands of California. *Aquatic Botany*, *91*, 123–129. https://doi.org/10.1016/j.aquabot.2009.03.006

Reigosa, M. J., & Pazos-Malvido, E. (2007). Phytotoxic effects of 21 plant secondary metabolites on *Arabidopsis thaliana* germination and root growth. *Journal of Chemical Ecology*, *33*, 1456–1466. https://doi.org/10.1007/s10886-007-9318-x

Ren, M. X., & Zhang, Q. G. (2007). Clonal diversity and structure of the invasive aquatic plant *Eichhornia crassipes* in China. *Aquatic Botany*, *87*, 242–246. https://doi.org/10.1016/j.aquabot.2007.06.002

Rice, E. L. (1984). *Allelopathy*. New York, NY: Academic Press.

Ruaux, B., Greulich, S., Haury, J., & Berton, J. P. (2009). Sexual reproduction of two alien invasive *Ludwigia* (Onagraceae) on the middle Loire River, France. *Aquatic Botany*, *90*, 143–148. https://doi.org/10.1016/j.aquabot.2008.08.003

Sakpere, A. A. M., Oziegbe, M., & Bilesanmi, I. A. (2010). Allelopathic effects of *Ludwigia decurrens* and *L. adscendens* subsp. *diffusa* on germination, seedling growth and yield of *Corchorus olitorious* L. *Notulae Scientia Biologicae*, *2*, 75. https://doi.org/10.15835/nsb224629

Santonja, M., Fernandez, C., Gauquelin, T., & Baldy, V. (2015). Climate change effects on litter decomposition: Intensive drought leads to a strong decrease of litter mixture interactions. *Plant and Soil*, *393*, 69–82. https://doi.org/10.1007/s11104-015-2471-z

Saxena, A., Singh, D. V., & Joshi, N. L. (1996). Autotoxic effects of pearl millet aqueous extracts on seed germination and seedling growth. *Journal of Arid Environments*, *33*, 255–260. https://doi.org/10.1006/jare.1996.0061

Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology and Evolution*, *8*, 275–279. https://doi.org/10.1016/0169-5347(93)90254-M

Smolders, A. J. P., Vergeer, L. H. T., van der Velde, G., & Roelofs, J. G. M. (2000). Phenolic contents of submerged, emergent and floating leaves of aquatic and semi-aquatic macrophyte species: Why do they differ? *Oikos*, *91*, 307–310. https://doi.org/10.1034/j.1600-0706.2000.910211.x

Stiers, I., Crohain, N., Josens, G., & Triest, L. (2011). Impact of three aquatic invasive species on native plants and macroinvertebrates in temperate ponds. *Biological Invasions*, *13*, 2715–2726. https://doi.org/10.1007/s10530-011-9942-9

Takeda, F., Nakano, K., Nishimura, O., Shimada, Y., Fukuro, S., Tanaka, H., … Inamori, Y. (2011). Allelopathic potential of *Potamogeton pusillus*

community against *Microcystis aeruginosa*. *Journal of Water and Environment Technology*, *9*, 21628.

Thouvenot, L., Haury, J., & Thiebaut, G. (2013). A success story: Water primroses, aquatic plant pests. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *23*, 790–803.

van Donk, E., & van de Bund, W. J. (2002). Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: Allelopathy versus other mechanisms. *Aquatic Botany*, *72*, 261–274. https://doi.org/10.1016/S0304-3770(01)00205-4

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., … Hulme, P. E. (2010). How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment*, *8*, 135–144. https://doi.org/10.1890/080083

Wang, J., Zhu, J. Y., Gao, Y. N., Liu, B. Y., Liu, S. P., He, F., & Wu, Z. B. (2013). Toxicity of allelochemicals released by submerged macrophytes on phytoplankton. *Allelopathy Journal*, *31*, 199–209.

Wu, Z. B., Gao, Y. N., Wang, J., Liu, B. Y., Zhou, Q. H., & Zhang, Y. Y. (2009). Allelopathic effects of phenolic compounds present in submerged macrophytes on *Microcystis aeruginosa*. *Allelopathy Journal*, *23*, 403–410.

Zhang, T. T., Wang, L. L., He, Z. X., & Zhang, D. (2011). Growth inhibition and biochemical changes of cyanobacteria induced by emergent macrophyte *Thalia dealbata* roots. *Biochemical Systematics and Ecology*, *39*, 88–94. https://doi.org/10.1016/j.bse.2011.01.004

Zhang, T. T., Zheng, C. Y., Hu, W., Xu, W. W., & Wang, H. F. (2010). The allelopathy and allelopathic mechanism of phenolic acids on toxic *Microcystis aeruginosa*. *Journal of applied phycology*, *22*, 71–77. https://doi.org/10.1007/s10811-009-9429-6

Zhou, S., Shaoa, Y., Gaoa, N., Dengc, Y., Qiaoa, J., Oua, H., & Denga, J. (2013). Effects of different algaecides on the photosynthetic capacity, cell integrity and microcystin-LR release of *Microcystis aeruginosa*. *Science of the Total Environment*, *463*, 111–119. https://doi.org/10.1016/j.scitotenv.2013.05.064

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Santonja M, Le Rouzic B, Thiébaut G. Seasonal dependence and functional implications of macrophyte–phytoplankton allelopathic interactions. *Freshwater Biol*. 2018;63:1161–1172. https://doi.org/10.1111/fwb.13124