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**BIOTIKUS INTERAKCIÓK SZUBMERZ ÉS EMERZ VÍZI
MAKROFITONOK KÖZÖTT**

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Az iskolavezető jóváhagyása

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1. ELŐZMÉNYEK ÉS CÉLKITŰZÉSEK

1.1 A vízi makrofitonok közötti kölcsönhatások

Az EU Víz Keretirányelv (VKI) jogszabály megszabja, hogy az EU országok felszíni vizeinek jó minőségűnek kell lennie adott fizikai, kémiai és biológiai mutatók alapján. Ennek megvalósítása komoly kihívások elé állítja a hidrobiológus szakembereket, mivel hazánkban számos állóvíz van, melynek minősége nem éri el az uniós elvárásokat. Felszíni vizeink a társadalom számára számos fontos szolgáltatást nyújtanak (ivóvíz, öntözés, fürdőzés, vízi sportok, halászat stb.). Ezeket közösen ökoszisztéma szolgáltatásoknak nevezzük, melyet alapvetően két tényező veszélyeztet: a növényi tápanyag túlkínálat (eutrofizáció) és a globális klímaváltozás. Ezen környezeti változások együttes hatására Magyarország vizes élőhelyeinek élőlényközösségei is jelentős átalakulásokon mennek keresztül: egyes fajok populációi gyorsan előretörnek, míg másoké hirtelen eltűnnek. A klímaváltozás és az eutrofizáció egymás hatását felerősítve fokozza a planktonikus algák (SCHEFFER & VAN NES 2007) vagy a felszínen úszó hínárnövények dominanciáját (SCHEFFER et al. 2003, SMITH 2014), továbbá az idegenhonos özönnövények invázióját (THUILLER et al. 2007). Az antropogén tevékenységek (pl. mezőgazdaság) miatt létrejött növényi tápanyag túlkínálat tovább erősíti az említett folyamatokat (PORTIELJE & ROIJACKERS 1995, ROIJACKERS et al. 2004), ezen túl a vízminőséget pozitívan befolyásoló őshonos alámerült hínárállományok visszaszorulását és pusztulását is okozza (PHILLIPS et al. 1978, 2016). Ennek hatására jelentős ökológiai károk jönnek létre, amelyek egyrészt az ember egészségére nézve számos káros következménnyel járnak (pl. cianobakteriális tömegprodukció invázió), másrészt az élőhelyek leromlását, a biodiverzitás csökkenését és a vízminőség romlását is eredményezik (JAKLIČ et al. 2020). Emiatt végső soron csökken a vizes élőhelyek társadalmi használati értéke. Az ökológiai károk elkerülése érdekében azonban fontos megismernünk, hogy az abiotikus környezeti tényezők hogyan befolyásolják a felszíni vizekben élő makrofitonok közötti biotikus kölcsönhatásokat.

A felszíni vizeinkben az elsődleges termelő szervezetek csoportjai (pl. vízinövények, algák) számos egymástól jól elkülönült ún. alternatív stabil állapotot tartanak fenn. Ezek az állapotok adott környezeti küszöbértéket átlépve újabb állapotra cserélődnek (regime shift), amely változás jelentős vízminőség-változással jár (PINTO & O'FARRELL 2014). Az elmúlt néhány évtizedben számos kutatás foglalkozott az úszó és az alámerült növények közötti kompetíciós viszonyok feltárásával, számításba véve az algák jelenlétét is. A nagy növényi tápelemkoncentráció (nitrogén, foszfor) a hullámnak kitett nyílt víztestekben alga dominanciát

eredményez nagy zavarossággal párosulva (SCHEFFER et al. 1993), míg ugyanez a tényező hullámzásmentes kis vizekben a szabadon úszó növényzet térhódítását okozhatja (SCHEFFER et al. 2003, PORTIELJE & ROIJACKERS 1995). Az úszó növényzet vastag takarója révén teljesen beárnyékolja a víztestet ami oxigénhiányhoz vezet, továbbá az alámerült (szubmerz) növények kipusztulásához vezet (MORRIS et al. 2003, PHILLIPS et al. 2016), továbbá állatok, kisebb makrogerinctelenek és a planktonikus algák pusztulását okozza (PINTO & O'FARRELL 2014). A globális felmelegedés az eutrofizációt támogatva tovább növeli az úszó hínárnövényzet inváziójának kockázatát (NETTEN et al. 2010). Ugyanakkor a sekélyebb víz kisebb tápanyag-koncentrációval (mezotróf-eutróf) párosulva elősegítheti a gyökerező szubmerz növények stabil dominanciájának kialakulását (VAN ZUIDAM & PEETERS 2013, SMITH 2014), aminek következtében nagyobb a víz átlátszósága, nagyobb az élőhely biodiverzitása és egyben a társadalmi használati értéke is. Terepi körülmények között több kutató megfigyelte, hogy sűrű alámerült hínárállomány fölött az úszó növények jelentősen kisebb mennyiségben vannak jelen (SCHEFFER et al. 2003, VAN ZUIDAM & PEETERS 2013). Ennek köszönhetően azt feltételezték, hogy az alámerült növények gátló hatást fejtenek ki a felettük elhelyezkedő úszó hínárállományra, melyet sikerült kísérletileg is bizonyítaniuk (SZABÓ et al. 2010, SZABÓ et al. 2022). Az alámerült növényzet ugyan széles tápelemkoncentráció-tartományban képes stabil dominanciát fenntartani, viszont egy környezeti küszöbérték felett vagy a planktonalgák, vagy pedig az úszó növényzet térhódításával dominanciájuk hanyatlani kezd. Az alámerült és az úszó növényzet közötti verseny kimenetelét a hínárállományon élő algabevonat (epifiton) is befolyásolhatja, mivel jelentősen leárnyékolja a szubmerz hínárnövényeket és csökkenti azok tápelem-felvételét. A megemelkedett tápelem-koncentráció és magasabb vízhőmérséklet, továbbá a növényevő makrogerinctelen szervezetek szintén módosíthatják az algák és a hínárnövények közötti verseny kimenetelét és elősegíthetik az idegenhonos hínárfajok invázióját.

1.2 Invazív hínárnövények terjedésének problémája és az invázió hatása a felszíni vizekben

Napjainkban a klímaváltozáson és eutrofizáción túl egy másik nagyon fontos biodiverzitást veszélyeztető tényezőként említhetjük az idegenhonos növények nagymértékű térhódítását (DUDGEON et al. 2006, STRAYER 2010). Számos felszíni vízben találkozhatunk invazív hínárnövényekkel, melyek megtelepedését, inváziós sikerességét több tényező befolyásolhatja. Az idegenhonos vízinövények megjelenése számos módon veszélyeztetheti a hazai hínárközösségeket. Az idegenhonos invazív hínárnövények a gyors növekedésük, a széleskörű elterjedésük valamint nagy fenotípusos plaszticitásuk révén súlyos gazdasági- és ökológiai károkat képesek okozni az újonnan meghódított területeken (GETSINGER et al. 2014, BRUNDU 2015). Gazdasági károk között főként a felszíni vizek eltorlaszolását említhetjük, mely kedvezőtlenül hat nem csak a hajózásra, de az ökoszisztéma szolgáltatásokra is. Ökológiai károk közé sorolhatjuk az abiotikus környezeti tényezők megváltozását, melyek magukba foglalják a fényintenzitás csökkenését a víz mélyebb rétegeiben (ROMMENS et al. 2003), az oldott oxigén koncentrációjának drasztikus csökkenését (JAMES et al. 1999, VILAS et al. 2017), valamint az anoxikus körülmények révén a biodiverzitás csökkenését (MORRIS et al. 2003). Ebből adódóan a hínárnövények azon tulajdonsága, hogy hogyan képesek a fent említett változásokra reagálni kulcsfontosságú lehet a vízinövények evolúciós sikerességét tekintve. Teljesen ismeretlenek azok a biológiai mechanizmusok, amellyel az őshonos és idegenhonos növényzet képes fenntartani stabil dominanciáját. Az sem feltárt még, hogy milyen környezeti és biológiai mechanizmusok irányítják az idegenhonos növényfajok felszíni vizekben való meghonosodását.

Jelenlegi adatok alapján kijelenthető, hogy az idegenhonos hínárnövényzet inváziója jelentős mértékben befolyásolja egy adott élőhelyre jellemző fajok sokféleségét és a funkcionális diverzitást (HEJDA & DE BELLO 2013). Közismert, hogy az idegenhonos fajok terjedése a fajok diverzitásának csökkenését okozza szárazföldi (HEJDA & DE BELLO 2013) és vízi környezetben egyaránt (VOJTKÓ et al. 2017, HUSSNER et al. 2021). Azáltal, hogy a területre jellemző őshonos fajok lecserélődnek, nagymértékben megváltoznak a különböző növényi jellegek (relatív növekedési ráta, specifikus levélfelület, levél szárazanyag-tartalom), melynek következtében megváltozik az ökoszisztéma működése és stabilitása (VILÀ et al. 2011).

1.3 Az értekezés főbb célkitűzései

Az értekezésben bemutatott tanulmányok mindegyike a felszíni vizeket érintő globális problémákkal foglalkozik a vízi makrofitonok közötti kölcsönhatásokat, a globális klímaváltozás hatását, illetve a növényi invázió témakörét kutatva. A hínárnövények közötti biotikus interakciók kutatása az ökoszisztéma szolgáltatások fenntartható használatához teremti meg az elméleti alapot. A laboratóriumi kísérletek és terepi adatelemzések során célunk volt, hogy feltárjuk az úszó és a szubmerz vízinövények közötti azon kölcsönhatásokat, melyek jelentős szerepet játszanak a vizes élőhelyek vízminőségének és természetközeli állapotának alakulásában.

Az értekezés célja feltárni, hogy:

- mekkora szerepet játszanak a makrofitonokon élő epifitikus algák és az őket fogyasztó makrogerinctelenek a szubmerz és emerz hínarak versenyében,
- a hazai vizekben már megjelent idegenhonos és honos fajok hogyan reagálnak különböző környezeti tényezők (fényintenzitás, hőmérséklet, tápanyagkoncentráció) változásaira,
- mely funkcionális jellegek teszik sikeressé az idegenhonos fajokat az őshonos fajokkal szembeni versenyben.

Ezen ismeretek birtokában a vízügyi ágazat részéről gyorsabb és hatékonyabb intézkedések tervezhetőek, amelyek segítségével hatékonyabban kontrollálható az idegenhonos és honos inváziós fajok terjedése, továbbá javítható a felszíni vizeink társadalmi hasznosíthatósági értéke. A kutatási kérdéseket laboratóriumi (mikrokozmosz) kísérletekből és terepi mérésekből származó adatok (Magyar Felszíni Vizek Adatbázisa) elemzésével és összevetésével válaszoljuk meg.

1.3.1 Az első tanulmány koncepciói és célkitűzései:

A felszíni vizekben az úszó és szubmerz növényzet egyaránt képes alternatív stabil állapotot fenntartani (SCHEFFER et al. 2003, SZABÓ et al. 2010, SZABÓ et al. 2022). Hipertróf körülmények között, a csatornáknban és kisebb tavakban a világszerte elterjedt érdes tócsagaz (*Ceratophyllum demersum*) gyakran alakít ki sűrű állományokat a vízfelszín alatt (LOMBARDO & COOKE 2003), azonban ugyanezen területeken sok esetben találkozhatunk púpos békalencsével (*Lemna gibba*), ami a felszínen úszó hínarak közé tartozik. Ugyanakkor a két növénycsoport közötti kompetíciós viszonyt a növények felületén élő epifitikus algák nagymértékben befolyásolhatják. Az epifitikus algák fontos szereplői a vizek litorális régiójának, hiszen nagymértékben meghatározzák a növények számára elérhető tápanyagok mennyiségét, illetve a fényviszonyokat (TÓTH 2013, LEVI et al. 2015). Ezen algák mennyiségét számos tényező befolyásolja, mint a fényviszonyok (CAO et al. 2017), a tápelem-koncentráció (YANG et al. 2020) vagy a hőmérséklet (KAZANJIAN et al. 2018) változása, illetve biotikus faktorok közül a forrásokért történő kompetíció (HANSSON 1988), valamint a herbivorok (CARPENTER & LODGE 1986). A csigák gyakran előfordulnak a vízi ökoszisztémákban és köztudott, hogy előszeretettel fogyasztják a növények felületén élő epifitikus algákat (PIP & STEWART 1976), melynek köszönhetően a növények több fényhez jutnak, ezáltal fokozzák azok növekedését (YE et al. 2019). A tanulmány fő célja tehát feltárni, hogy mekkora szerepet játszanak a makrofitonokon élő epifitikus algák és az őket fogyasztó makrogerinctelenek a szubmerz és úszó növények közötti versenyben.

1.3.2 A második tanulmány koncepciói és célkitűzései:

Napjainkban az idegenhonos fajok megjelenése és a globális klímaváltozás két olyan tényező, melyek a biodiverzitást nagymértékben veszélyeztetik. A klímaváltozás a környezetvédelem egyik legnagyobb problémája, hiszen édesvizekben jelentősen elősegíti az eutrofizáció folyamatát (JEPPESEN et al. 2010), továbbá hatására az őshonos hínárnövények képtelenek felvenni a versenyt az idegenhonos növényekkel szemben (REJMANEK & RICHARDSON 1996). A globális felmelegedés fokozza a szabadon úszó növényzet dominanciáját (PEETERS et al. 2013), melynek kialakulását a nagy tápelem-terhelés még inkább elősegíti (SCHEFFER et al. 2003, SMITH 2014, SZABÓ et al. 2022), ezáltal csökkentve a szubmerz növények számára elérhető fény mennyiségét. A tanulmányban felhasznált két alámerült növény a karolinai tündérhínár (*Cabomba caroliniana*) és a füzéres süllőhínár

(*Myriophyllum spicatum*). A *Cabomba* egy gyors növekedésű szubmerz növény, mely Dél-Amerikában és az Egyesült Államok délkeleti részén őshonos (ØRGAARD 1991), azonban az elmúlt években nagy számban jelentették európai megjelenését, köszönhetően annak, hogy az akvarisztikában rendkívül közkedvelt és széles körben alkalmazott növényé vált. A *Myriophyllum* egy hasonló életformájú növény, mely Európában, Ázsiában és Észak Afrikában őshonos (PATTEN 1954). A két növény gyakran megtalálható együtt, ugyanazon élőhelyen, így kompetíciós kölcsönhatás is kialakul közöttük. Ezért a tanulmányunk célja volt, hogy feltárjuk a különböző környezeti tényezők (fényintenzitás, hőmérséklet, tápelemkoncentráció) kombinált hatását a két növény bizonyos jellegeire nézve. További célunk volt, hogy ezen eredmények alapján felmérjük, hogyan változik meg a két növény közötti kompetíció a változó környezeti tényezők hatására.

1.3.3 A harmadik tanulmány koncepciói és célkitűzései:

A biológiai invázió következtében egy adott területre jellemző növények funkcionális sokféleségének csökkenése a vízinváziók esetében még mindig kevésbé tanulmányozott. A legtöbb újonnan megtelepedett invazív idegenhonos vízinvázió rendkívül gyorsan képes terjedni, ezáltal súlyos károkat okozva az adott területen (BRUNDU 2015, ZHAN et al. 2017). Az idegenhonos növényfajok terjedése - szárazföldi (HEJDA & DE BELLO 2013) és vízi élőhelyeken (HUSSNER et al. 2021) egyaránt - kedvezőtlenül befolyásolja a terület biodiverzitását. Az új invazív növényfajok elterjedése alapjaiban képes megváltoztatni az ökoszisztéma működését és stabilitását (VILÀ et al. 2011). Az idegenhonos vízinváziókra jellemző magasabb növekedési ráta, a hatékonyabb terjedési mechanizmusok (HUSSNER et al. 2021, TASKER et al. 2022), a nagyobb fenotipikus plaszticitás (FLEMING & DIBBLE 2015, SZABÓ et al. 2019), az árnyéktűrő képesség (SZABÓ et al. 2020), az erőforrások jobb felhasználása (LUKÁCS et al. 2017) mind hozzájárulnak az idegenhonos fajok terjedésének sikerességéhez az őshonos fajokkal szemben. Ezen tulajdonságoknak köszönhetően ezek a növények gyakran hoznak létre monospecifikus állományokat az adott élőhelyen, ami miatt megváltozik az élőhely szerkezete. Ennek a tanulmánynak a célja tehát, hogy feltárjuk, hogyan változtatja meg a biológiai invázió az őshonos hínárnövény-közösségek funkcionális szerkezetét és sokféleségét.

2. ANYAGOK ÉS MÓDSZEREK

2.1 Első tanulmány: Az epifitikus algák és csigák szerepe az alámerült és szabadon úszó hínárnövények stabil állapotaiban

A kísérletben felhasznált púpos békalencsét (*Lemna gibba*) és érdes tócsagazt (*Ceratophyllum demersum*) valamint a nagy pocsolyacsigát (*Radix labiata*) a Nyíregyháza mellett húzódó Igrice csatornából gyűjtöttük. A kísérletet laboratóriumi körülmények között végeztük, melynek megkezdése előtt a növényeket BARKO & SMART (1985) által kidolgozott tápoldaton inkubáltuk. Ehhez hozzáadtunk 5 mg L⁻¹ nitrogént (NaNO₃) és 1 mg L⁻¹ foszfort (K₂HPO₄), illetve a tápoldat mikroelem tartalmát 0,1 mL L⁻¹ Tropica oldat hozzáadásával biztosítottuk. A növényeket 14 napon keresztül 20 literes tenyészedényekben neveltük 220 μmol m⁻² s⁻¹ megvilágításon, 16:8 (fény:sötét) óra fotoperiódussal, 25 °C-on.

A kísérlet során az inkubációs körülmények változatlanok maradtak, csupán a tenyészedények mérete változott, ebben az esetben 2 literes műanyag akváriumokat alkalmaztunk, melyek oldalait befedtük fekete fóliával a zavaró fényhatás kiküszöbölése érdekében. Az oldathoz 10 mL L⁻¹ algaszuszpenziót adtunk, melyet a tócsagaz felületéről öblítettünk a tápoldatba. Az akváriumok közepén elhelyezkedő PVC csőbe 100 mg nedves tömegű békalencsét helyeztünk (SZABÓ et al. 2003). A békalencsék túlzásfóltóságát és az emiatt bekövetkező intraspecifikus kompetíciót elkerülve a PVC cső méretét mindig 1 cm-rel nagyobb átmérőjűre cseréltük, ahogy a békalencsék szaporodtak. Az összesen 5 féle kezeléssel két különböző kontroll kultúrát alkalmaztunk: egy algamenteset és egy algával kezeltet. Az algával kezelt kontroll kultúra tenyészedényeiben lévő víz felszínét műanyag lappal takartuk le. A békalencse-tócsagaz kevert kultúrákba 0 g és 10 g nedves tömegű tócsagaz hajtást helyeztünk (KOLESZÁR et al. 2022, 1. ábra). Az algával kezelt békalencse kultúrák és a békalencse-tócsagaz kevert kultúrák esetében további kezelésként akváriumonként 3 db (egyenként 1,5-3 g) nagy pocsolyacsigát alkalmaztunk az algásodás minimalizálása érdekében. A csigák elvándorlásának és a békalencse fogyasztás megakadályozásának érdekében ragasztószalagot helyeztünk az akváriumok és a PVC cső széleire. A kultúrákat alacsony és magas (0,5 és 5 mg N L⁻¹, NaNO₃) nitrogénkoncentráción és állandó foszforkoncentráción (1 mg P L⁻¹, K₂HPO₄) neveltük. Minden kezelést háromszoros ismétlésben végeztünk el. A békalencse-tócsagaz (5 mg N L⁻¹) kevert kultúrák tápoldataiból vízmintát vettünk melynek megmértük a pH-ját, majd a szűrést követően (0,45 μm pórusátmérő) meghatároztuk a PO₄³⁻-P tartalmát valamint az oldott szervesetlen nitrogén (NO₃⁻-N, NH₄⁺-N) koncentrációját (MSZ ISO 7150-1:1992, MSZ EN ISO

6878:2004, MSZ 1484-13:2009). A békalencse nedves tömegét a 4., 8. és 12. napon mértük le, majd számoltunk belőle növekedési rátát (RGR) a következők szerint: $RGR = (\ln W_t - \ln W_0)/t$, ahol a W_0 a kiindulási nedves tömeg, W_t pedig a "t" napon mért nedves tömeg (LANDOLT & KANDELER 1987). A 12. napon lemértük a növények száraztömegét is, valamint a tenyészedényekben lévő algák biomasszáját, melyet a növények felületéről és az akváriumok oldaláról ecset segítségével a tápoldatba mostunk, majd algaszűrőn (5-8 μm pórusátmérő) szűrtük, végül a szűrőpapírt szárítószekrényben 80 °C-on 48 órán keresztül szárítottuk. Az algák és makrofitonok nitrogén tartalmát Vario Max Cube segítségével határoztuk meg, majd a növények nitrogén felvételét (mg) a kiindulási alminták és az utolsó napon mért biomasszáik nitrogéntartalmának különbségéből számítottuk ki.

A laboratóriumi eredményeket összevetettük az Országos Vízügyi Főigazgatóság (<https://www.ovf.hu/>) honlapjáról gyűjtött adatokkal, valamint 349 olyan méréssel, amely 17 különböző európai országból származik 1994 és 2011 között, olyan területekről, ahol a púpos békalencse (*L. gibba*) és/vagy az érdes tócsagaz (*C. demersum*) megtalálható.

A független és függő változók közötti interakciókat általánosított lineáris modell (GLM) segítségével vizsgáltuk. A változók normál eloszlását és a szóráshomogenitást Levene-teszttel ellenőriztük. A csigákkal történő kezelések közötti különbségeket (békalencse biomassza, békalencse- és tócsagaz növekedési ráta, alga száraztömeg, pH, nitrogénfelvétel) páronkénti összehasonlítással (PC) elemeztük. A makrofitonok (békalencse, tócsagaz) és a fitoplankton klorofill-a közötti kapcsolatot, valamint a békalencse és tócsagaz borítása közötti kapcsolatot Fisher egzakt teszttel elemeztük. A statisztikai elemzéseket SPSS 16.0 programmal végeztük el.

2.2 Második tanulmány: Árnyéktűrő képesség, mint kulcstényező a szubmerz *Cabomba caroliniana Myriophyllum spicatum*-mal szembeni inváziós sikerében

A kísérletben felhasznált karolinai tündérhínárt (*Cabomba caroliniana*) a hévízi tóból, a füzéres süllőhínárt (*Myriophyllum spicatum*) a Keleti-főcsatornából gyűjtöttük. A növényeket a kísérlet megkezdése előtt tenyészedényekben előinkubáltuk, melyeket BARKO & SMART (1985) leírása alapján elkészített tápoldaton neveltük. A növényeket két különböző tápanyagkoncentráción (eutróf (0,5 mg N L⁻¹ és 0,05 mg P L⁻¹) és hipertróf (2 mg N L⁻¹ és 0,2 mg P L⁻¹) neveltük NH₄NO₃ és K₂HPO₄ hozzáadásával. A tápoldat mikroelem tartalmát 0,1 mL L⁻¹ TROPICA törzsoldat felhasználásával biztosítottuk. Az előinkubáció 14 napon keresztül tartott a következő feltételek mellett: 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$ megvilágítás, 16:8 (fény:sötét) óra fotoperiódus,

24,5 ± 0,5 °C. A kísérlet megkezdése előtt a növények felületén lévő vizet centrifuga (Hajdu 407.6 centrifuga, 1400 RPM, 10 másodperc) segítségével eltávolítottuk, majd mindkét faj hajtásaiból almintákat vettünk, melynek lemértük a nedves-, illetve a száraz tömegét is.

A kísérletben mindkét növényből 6 db (11-14 cm-es) apikális hajtásrészt helyeztünk nagyjából hasonló tömegben (7,4 ± 0,2 g nedves tömeg) egymástól elkülönítve 2 literes akváriumokba, melynek oldalát fekete fóliával fedtük be meggátolva a fény áthaladását az oldalakon keresztül. Az akváriumokba a fent ismertetett módon készítettük el a tápoldatot, majd a pH-t 7,3-ra állítottuk be. A kísérlet során két különböző tápanyag-koncentrációt (0,5 mg N L⁻¹, 0,05 mg P L⁻¹; 2 mg N L⁻¹ és 0,2 mg P L⁻¹), négy különböző fényintenzitást (22–28, 52–82, 170–290 és 260–330 μmol m⁻² s⁻¹) és két eltérő hőmérsékletet (21,5 és 27,5 ± 0,5 °C) állítottunk be. A kísérlet 8 napon keresztül tartott. A tápanyag-utánpótlás kétnaponta, azaz a 2., 4. és 6. napon történt. Minden kezelést (2x2x2x4=32) háromszoros ismétlésben végeztünk el, ami azt jelenti, hogy összesen 96 akváriummal dolgoztunk.

Minden akváriumból 3 db levelet vettünk a növényekről (96x3=288) PÉREZ-HARGUINDEGUY et al. (2016) protokollja alapján, majd megmértük a levelek felületét LI-3000 Leaf Area Meter + LI-3050C Transparent Belt Conveyor Accessory műszerek segítségével. Ezt követően lemértük a teljes szubmerz növény és a levágott három levél nedves tömegét és szárítószekrényben 80 °C-on 48 órán keresztül történő szárítás után a száraz tömegét is. Ezen értékek alapján számítottunk relatív növekedési rátát (RGR), specifikus levélfelületet (SLA) és levél szárazanyagtartalmat (LDMC). Az RGR számítása az első tanulmányban részletezett módon történt. A növények specifikus levélfelületét a következőképpen számoltuk: SLA = (LA/W mm² mg⁻¹), ahol LA a levélfelület és W a levelek száraz tömege (GARNIER et al. 2001). A levelek szárazanyagtartalmát a következőképpen számítottuk: LDMC = (DW/WW mg g⁻¹), ahol DW levelek száraz tömege és WW a levelek nedves tömege (GARNIER et al. 2001). A szárított növények nitrogén és szén tartalmát a Vario Max Cube elemental analyzer műszer segítségével határoztuk meg. Kiszámítottuk a fény (T), a hőmérséklet (T) és tápelemkoncentráció (N) hatására történt RGR, SLA és LDMC értékek változásaiból mindkét faj plaszticitási indexeit (Pi_L, Pi_T Pi_N) VALLADARES et al. (2002, 2005) módszere alapján, miszerint: PI = (maximum átlag-minimum átlag)/maximum átlag. Az eredmény 0 és 1 közé eső szám, ahol „0” jelentése: nincs plaszticitás; „1” jelentése: maximális plaszticitás.

A változók normál eloszlását Kolmogorov-Smirnov teszttel ellenőriztük. A változók közötti kölcsönhatásokat és azok szignifikancia szintjét általánosított lineáris modell (GLM) segítségével vizsgáltuk. A fajok (*C. caroliniana*, *M. spicatum*) közötti különbségeket páronkénti összehasonlítással (PC) elemeztük. A független változók függő változókra gyakorolt hatását

varianciaelemzéssel (ANOVA) elemeztük. A statisztikai elemzéseket SPSS 16.0 programmal végeztük el.

2.3 Harmadik tanulmány: A fajon belüli jellegek variabilitása releváns az őshonos és idegenhonos vízínövény-társulások funkcionális összetételbeli különbségének értékelése szempontjából.

A kutatás helyszínéül a Hévízi-csatorna szolgált, amely egy természetes kis folyó, állandó vízszinttel és körülbelül 20 °C-os évi átlag középhőmérséklettel rendelkezik. Az 1980-as években a csatorna felső szakaszán illegálisan termesztettek akváriumi növényeket, aminek köszönhetően az idegenhonos invazív növények sűrű társulásokat alkothatnak szemben a csatorna alsó szakaszával, ahol az őshonos vízínövényzet az uralkodó. A mérséklet égvői területeken az idegenhonos trópusi növények áttelelése szempontjából egy nagyon fontos tényező a téli időszak átvészélése. Azok az édesvízi élőhelyek, melyek magasabb vízhőmérséklettel rendelkeznek, kitűnő feltételeket biztosítanak a hidegérzékeny vízínövények számára az átteleléshez. Az ilyen területek lehetőséget biztosítanak a trópusi, szubtrópusi fajok számára a vegetatív formában történő áttelelésre, vagy elősegíthetik a magok és/vagy rügyek fennmaradását, aminek köszönhetően a tavasz beköszöntével még az őshonos vízínövények elterjedése előtt sűrű vegetációkat alkothatnak. Európai viszonylatban ezek a területek a fő helyszínei a vízínövények inváziójának (ŠAJNA et. al 2007, HUSSNER et al. 2014, LUKÁCS et. al 2016), amelynek köszönhetően ideális kutatási területet biztosítanak az idegenhonos és őshonos vízínövények közötti funkcionális diverzitásbeli különbségek tanulmányozásához.

Összesen 20 db (2x2 m-es) kvadrátot helyeztünk el a csatorna mentén, melyből 10 db-ot a Hévízi-csatorna meleg vizű, felvízi szakaszán és 10 db kvadrátot a csatorna hidegebb vizű, alvízi szakaszán helyeztünk el. Erre az idegenhonos és őshonos fajok egyenlőtlen eloszlása miatt volt szükség. A kvadrátokat a csatorna középvonalában (sodorvonal) helyeztük el, azokban rögzítettük a fajok %-os borítását, 2017. szeptember 9-11 között. A borításbecslés során figyelembe vettük, hogy a hínárnövények esetében kifejezett az egyes szintek közötti átfedés, ezért a kvadrátok teljes borítása meghaladhatta a 100%-ot. A becslést követően minden kvadrátban, minden fajból 5 db levélmintát gyűjtöttünk a funkcionális jellegek meghatározásához, majd begyűjtöttük a teljes növényzetet a nedves fitomassza meghatározása érdekében. Minden kvadrátban megmértük a víz hőmérsékletét, pH és vezetőképesség értékeit Hach Lange HQ40D multiparaméteres szondákkal, valamint vízmintát vettünk az összes nitrogén- és foszfortartalom meghatározásához. Lemértük a levelek nedves- és száraztömegét, valamint levélfelületét is. A száraztömeg értékeket szárítószekrényben 80°C-on 48 órán keresztül

történő szárítás után mértük le, a levélfelület meghatározásához pedig a LI-3000 Leaf Area Meter + LI-3050C Transparent Belt Conveyor Accessory műszereket használtuk. A specifikus levélfelület (SLA) és a levél szárazanyagtartalom (LDMC) meghatározása a második tanulmányban is ismertetett módon történt.

Kiszámoltuk a közösségi súlyozott átlagos jellegértékeket (GARNER et al. 2004) melyek az abundancia által súlyozott jellegek átlagértékei. Számoltunk funkcionális diverzitás értékeket, melynek során figyelembe vettük a funkcionális gazdagságot, a funkcionális egyenletességet és a funkcionális divergenciát (MASON et al. 2005, VILLÉGER et al. 2008). A funkcionális gazdagság nem más, mint a fajok által kitöltött „jellegtér”; a funkcionális egyenletesség a jellegértékek eloszlását mutatja meg az adott területen; a funkcionális divergencia pedig annak a mérőszáma, hogy az abundancia eloszlása mennyire maximalizálja a közösség funkcionális karaktereinek különbözőségét (MASON et al. 2005). A funkcionális gazdagság, a funkcionális egyenletesség és a funkcionális divergencia értékeket a fajszintű jelleg valószínűségi eloszlásokból (TPDs) számítottuk CARMONA et al. (2016, 2019) módszere alapján. Az elemzéshez két féle módszert alkalmaztunk, az ún. global és optimal pooling módszert. Global pooling módszer esetében egyetlen jelleg valószínűségi eloszlást (TPDs) vizsgáltunk minden egyes fajra. Optimal pooling módszer esetében a kijelölt kvadrátokat csoportokra osztottuk Gaussian finite mixture model alapján, majd így vizsgáltuk a jelleg valószínűségi eloszlásokat minden egyes csoportra BOTTA-DUKÁT & LUKÁCS (2021) módszere szerint. A funkcionális diverzitás elemzése R programcsomag használatával, ks (DUONG et al. 2022), mclust (SCRUCCA et al. 2016) és TPD (CARMONA 2019) csomag segítségével történt. Az idegenhonos és őshonos fajok közösségi súlyozott átlagait, valamint a funkcionális diverzitás indexeit Wilcoxon teszt segítségével hasonlítottuk össze. Az ábrákat és a Wilcoxon tesztet Origin Pro 2023 program alkalmazásával készítettük.

3. EREDMÉNYEK

3.1 Első tanulmány: Az epifitikus algák és csigák szerepe az alámerült és szabadon úszó hínárnövények stabil állapotaiban

Mind az algák mind a tócsagaz jelenléte szignifikánsan csökkentette a békalencse biomasszáját. A tócsagaz és az algák békalencsére gyakorolt együttes gátló hatásból 20%-ot az algák okoztak. A békalencse-tócsagaz kevert kultúrákban alacsony nitrogénkoncentráción ($0,5 \text{ mg N L}^{-1}$) nem volt különbség a békalencse növekedési rátáját tekintve a csigákkal kezelt és a csigák nélküli akváriumok között, azonban magas (5 mg N L^{-1}) nitrogénkoncentráción a csigák jelenléte fokozta a békalencse növekedését. Ugyanezt a serkentő hatást tapasztaltuk tócsagaz esetében is, ugyanis csigák jelenlétében a tócsagaz biomasszája jelentősen nagyobb volt, mint csigák nélkül. A békalencse-tócsagaz kevert kultúrákban a csigák 67-86%-al csökkentették az algák biomasszáját. Csigák jelenlétében jelentősen ($10,01 \pm \text{SE } 0,027$ -ről $9,63 \pm \text{SE } 0,036$ -ra) csökkent a tápoldat pH értéke. A kísérlet 6. napjára csigák nélkül a tápoldat $\text{NO}_3\text{-N}$ koncentrációja 97%-al, $\text{PO}_4^{3-}\text{-P}$ koncentrációja pedig 70%-al volt alacsonyabb, mint csigákkal. Csigák jelenlétében magasabb volt a békalencse és a tócsagaz szöveti nitrogénkoncentrációja (8 és 15%-al), valamint 128%-al fokozódott a békalencsék és 194%-al a tócsagaz nitrogénfelvétele, ugyanakkor az alga biomassza 96%-os csökkenést mutatott. A terepi mérések szerint nem találtunk szignifikáns kapcsolatot sem a tócsagaz borítás és a fitoplankton klorofill-a mennyisége, sem a békalencse borítás és a fitoplankton klorofill-a mennyisége között. Azonban az elemzések alapján a fitoplankton klorofill-a mennyisége azokon a területeken volt a legnagyobb, ahol alacsony volt a békalencsék vagy a tócsagaz borítása. Amint a fitoplankton klorofill-a koncentráció $30 \mu\text{g L}^{-1}$ -nél magasabb volt, ott a békalencsék és a tócsagaz borítása kevesebb volt, mint 3%. 715 európai adat elemzése alapján a Fisher egzakt teszt szignifikáns ($P < 0,001$) kapcsolatot mutatott a békalencse és a tócsagaz borítás között, mely kapcsolat erősen negatív, ebből adódóan minél nagyobb a békalencse borítás egy adott területen, annál kisebb a tócsagaz mennyisége. Ugyanakkor az eredmények azt mutatták, hogy a tócsagaz és a csigák mennyisége között pozitív korrelációs kapcsolat áll fenn, mivel a nagyobb tócsagaz denzitás mellett nagyobb volt a csigák egyedszáma.

A strukturális egyenletmodellek eredményei alapján a víz nitrogénkoncentrációja serkentette mind a békalencsék, mind az epifitikus algák biomasszáját, ugyanakkor a nitrogénkoncentráció $0,5 \text{ mg L}^{-1}$ -ről 5 mg L^{-1} -re történő emelése csökkentette a tócsagaz növekedését. Az epifitikus algák növelték a tápoldat pH-ját, ugyanakkor csökkentették a tápoldat

szervetlen nitrogén és foszfor koncentrációját, valamint a békalencsék biomasszáját. A csigák jelenléte csökkentette az algák biomasszáját és a tápoldat pH-ját, valamint erősen fokozta a tócsagaz és a békalencse növekedését.

3.2 Második tanulmány: Árnyéktűrő képesség, mint kulcstényező a szubmerz *Cabomba caroliniana* *Myriophyllum spicatum*-mal szembeni inváziós sikerében

A kísérlet során a magasabb hőmérséklet ($27,5 \pm 0,5$ °C) alacsony fényintenzitáson (22–28 és 52–82 $\mu\text{mol m}^{-2} \text{s}^{-1}$) szignifikánsan csökkentette mindkét faj növekedését. A kísérlet egészét tekintve a *Cabomba* jelentősen nagyobb növekedési rátát mutatott a *Myriophyllum*-hoz viszonyítva, azonban ez a különbség alacsony fényintenzitáson (22–28 és 52–82 $\mu\text{mol m}^{-2} \text{s}^{-1}$) még nagyobb volt. Az erősen megvilágított körülmények között (170–290 és 260–330 $\mu\text{mol m}^{-2} \text{s}^{-1}$) viszont a fajok növekedési ráta értékei nem különböztek egymástól. A két faj közül a *Cabomba* szignifikánsan nagyobb specifikus levélfelület értékekkel rendelkezett, mint a *Myriophyllum*. A fényintenzitás és a hőmérséklet is befolyásolta a *Cabomba* specifikus levélfelület értékeit, azonban a *Myriophyllum* esetében sem a fényintenzitás, sem a hőmérséklet sem pedig a tápanyagkoncentráció nem volt rá hatással. Levél szárazanyagtartalom tekintetében a *Myriophyllum* rendelkezett magasabb értékekkel. A nitrogén:szén arányt vizsgálva elmondható, hogy a *Myriophyllum* értékei nagyobbak voltak, mint a *Cabomba*-é és ez a különbség kisebb fényintenzitáson (22–28 és 52–82 $\mu\text{mol m}^{-2} \text{s}^{-1}$) még nagyobb volt. Alacsony fényintenzitáson és magas hőmérsékleten a *Cabomba* nitrogénfelvétele több mint háromszor magasabb volt, mint a *Myriophyllum*-é. Minden vizsgált változót figyelembe véve a *Cabomba* jelentősen nagyobb fenotipikus plaszticitással rendelkezett a *Myriophyllum*hoz képest.

3.3 Harmadik tanulmány: A fajon belüli jellegek variabilitása releváns az őshonos és idegenhonos vízínövénytársulások funkcionális összetételbeli különbségének értékelése szempontjából.

A tanulmány helyszínéül szolgáló Hévízi-csatorna alsó, hidegebb vízhőmérsékletű szakaszán az őshonos vízínövényzet (*Ceratophyllum demersum*, *Hydrocharis morsus-ranae*, *Lemna minor*, *Nuphar lutea*, *Spirodela polyrhiza*) volt uralkodó. Idegenhonos növények ezen a területen nem fordultak elő. A felső, melegebb vízhőmérsékletű szakaszon ezzel szemben az idegenhonos fajok voltak túlsúlyban (*Cabomba caroliniana*, *Hygrophila diformis*, *Nymphaea lotus*, *Nymphaea rubra*, *Rotala rotundifolia*, *Vallisneria americana*, *Vallisneria spiralis*), az őshonos fajok pedig nem fordultak elő ezen a területen. A csatorna felső szakaszán jóval magasabb volt az összes foszforkoncentráció, valamint a víz vezetőképessége, mint az alsó szakaszon; a pH-ja viszont alacsonyabb volt. Az őshonos fajok uralta területeken 64%-al kisebb nedvestömeg biomasszát mértünk, mint a csatorna felső szakaszán. Ugyanakkor az itt előforduló őshonos fajok levélfelülete (LA) nagyobb volt, specifikus levélfelületük (SLA) pedig kisebb, mint az idegenhonos fajoké. Levél szárazanyag tartalom (LDMC) tekintetében nem találtunk különbségeket az idegenhonos és őshonos fajok között. Az eredmények értelmezésekor a global és az optimal pooling módszer között jelentős eltéréseket tapasztaltunk. A global pooling módszer jellemzően túlbecsülte a funkcionális gazdagság értékeit függetlenül a különböző jellegektől (LA, SLA, LDMC) és közösségtípusoktól, ugyanakkor idegenhonos fajok esetében az SLA és LDMC funkcionális divergencia értékeit jellemzően alábecsülte. A funkcionális egyenletességet vizsgálva a két módszer között nem volt lényeges különbség. A global pooling módszer eredményei szerint az idegenhonos fajok levélfelületi funkcionális gazdagsága és egyenletessége, valamint a levél szárazanyag tartalom és specifikus levélfelület funkcionális egyenletessége szignifikánsan nagyobb volt, míg a levélfelület funkcionális divergenciája és a specifikus levélfelület funkcionális gazdagsága alacsonyabb volt, mint az őshonos fajoké. Az optimal pooling módszer alkalmazásával ezen hat tényező közül mindössze négy esetben találtunk jelentős eltéréseket. Ezek alapján az idegenhonos fajok magasabb levélfelületi funkcionális gazdagsága és egyenletessége, valamint a levél szárazanyag tartalom magasabb funkcionális egyenletessége és az idegenhonos fajok alacsonyabb levélfelületi funkcionális divergencia értékei egyeztek meg a global pooling módszer eredményeivel.

4. KÖVETKEZTETÉSEK ÉS JAVASLATOK

Ennek az értekezésnek a fő célkitűzése az alámerült és felszínen úszó hínárnövények közötti biotikus kölcsönhatások vizsgálata és bemutatása volt. Ezeket az interakciókat főként az első tanulmány keretein belül vizsgáltuk, ahol sikerült több kölcsönhatást is kimutatni az úszó- és szubmerz növények, valamint az epifitikus algák és csigák között az abiotikus tényezők változásának nyomon követésével (pH érték, tápelem-koncentráció). A kísérletben a két különböző életformájú növénycsoportot a szabadon úszó békalencse (*Lemna gibba*) és az alámerült nem gyökerező érdes tócsagaz (*Ceratophyllum demersum*) képviselte. Egy korábbi tanulmány keretein belül (SZABÓ et al. 2022) már vizsgáltuk, hogy ezek a növénycsoportok milyen körülmények között képesek fenntartani stabil dominanciájukat a másik növénycsoporttal szemben. SZABÓ et al. (2022) eredményei alapján azt a következtetést vontuk le, hogy kisebb nitrogénkoncentráció (<3 mg N L⁻¹) mellett tócsagaz dominancia alakul ki, míg nagyobb nitrogénkoncentráción (>5 mg N L⁻¹) békalencse dominancia jellemző egy adott víztestben. Azonban a disszertáció keretein belül bemutatott első tanulmányban megvizsgáltuk, hogy a nagy pocsolyacsiga jelenléte a rendszerben milyen hatással van az algaközösségek biomasszájára, hogyan változtatja meg a tápoldat nitrogén- és foszfortartalmát, valamint az algák mekkora szerepet játszanak a békalencsék növekedésgátlásban. A második és harmadik tanulmányban nagyobb hangsúlyt fektettünk a klímaváltozás által megváltoztatott abiotikus környezeti tényezők (fényintenzitás, hőmérséklet, tápelemkoncentráció) idegenhonos és őshonos vízinövény fajokra gyakorolt hatásainak vizsgálatára, valamint a növényi invázió által okozott ökoszisztémát érintő problémákra és változásokra.

Végezetül szeretném összefoglalni az értekezés keretein belül bemutatott három tanulmányban milyen kölcsönhatásokat sikerült kimutatni, valamint ezek eredményei alapján milyen következtetéseket vonhatunk le, illetve milyen javaslatokat tehetünk.

Az értekezésben bemutatott tanulmányok eredményei szerint az epifitikus algák és a tócsagaz jelenléte egyaránt gátolta a békalencsék növekedését, ami főként a tápanyagelvonás miatt következhetett be, ugyanis a tócsagaz-alga komplex csigák nélkül jóval gyorsabban csökkentette a tápoldat nitrogén és foszforkoncentrációját, mint csigák jelenlétében. Ugyanakkor az algák és a tócsagaz is képes fokozni a tápoldat pH értékét, hiszen a fotoszintézisükhöz szükséges HCO_3^- felvétele miatt hidroxidion (OH^-) szabadul fel (PEDERSEN et al. 2013). A víz megemelkedett pH értéke csökkenti az anionok (NO_3^- , PO_4^{3-}) felvehetőségét (ULLRICH-EBERIUS 1981) valamint hozzájárul a foszfát, a vas és a mangán kicsapódásához (OTSUKI & WETZEL 1972, STUMM & MORGAN 1995). A tanulmányban laboratóriumi körülmények között kimutatott nagy (> 10) pH gyakran megtalálható terepi körülmények között is azokon a területeken, ahol az alámerült növények (pl. *Ceratophyllum*, *Elodea*) sűrű állományokat alkotnak (FRODGE et al. 1990). A csigák képesek voltak fokozni a békalencsék növekedését, ami egy közvetett hatásnak tudható be, hiszen a hatékony algalegelés miatt csökken az algák fotoszintetikus aktivitása és tápelem-felvétele. Ennek következtében a tápoldat pH értéke csökken, tápelem-koncentrációja (N, P) viszont emelkedik. Ezen túlmenően a csigák a szubmerz növények növekedését is elősegítették, hiszen a növények felületén élő algabevonat egyfajta akadályt képez a növények számára (SAND-JENSEN 1977), melyek így képtelenek felvenni a szükséges mennyiségű tápanyagot. Az epifita algák gátló hatása nagy tápanyag-koncentráció mellett erősebb volt. Alacsony tápanyag-koncentráción az algák békalencsék növekedésére gyakorolt gátló hatása nem volt jelentős, hiszen ebben az esetben az alámerült tócsagaz olyan mértékben képes elvonni a tápanyagot a környezetéből, amely már alapvetően megakadályozza az epifitikus algák elszaporodását. Ezek alapján azt a következtetést vonhatjuk le, hogy a szubmerz és úszó növények közötti kompetíció kimenetelét, valamint a növénycsoportok (úszó és szubmerz) stabil állapotait az algák és a csigák jelentős mértékben képesek befolyásolni. A csigák annak ellenére, hogy elősegítik a szubmerz növények növekedését, mégis destabilizálják az alámerült növények úszó növényekkel szembeni stabil állapotát, hiszen az algák fogyasztásával csökken az alámerült növények úszó növényekre gyakorolt negatív hatása. Ennek köszönhetően az úszó növények már alacsonyabb tápelem-koncentráció mellett is képesek kialakítani a stabil dominanciát a szubmerz növények fölött.

Napjainkban a klímaváltozás különböző hatásainak köszönhetően számos tényező megváltozik a víz mélyebb rétegeiben, amelyhez való alkalmazkodás a szubmerz növények szempontjából kulcstényezőnek bizonyulhat. Eredményeink szerint az idegenhonos *Cabomba caroliniana* specifikus levélfelülete (SLA) jelentősen nagyobb volt, mint a *Myriophyllum spicatum* SLA értékei, a levél szárazanyag tartalom tekintetében pedig ellenkezőleg, a *Cabomba*

értékei voltak alacsonyabbak. A magasabb SLA és az alacsonyabb LDMC pedig arra enged következtetni, hogy egységnyi száraz biomasszából a *Cabomba* jelentősen nagyobb levélfelületet képes előállítani, amely hatékonyabb fényelnyelést és fotoszintézist eredményez. Ezen túlmenően több tanulmány is alátámasztja (LAKE & LEISHMAN 2004, HAMILTON et al. 2005), hogy a magasabb SLA elősegítheti a növények invázióját. Továbbá a *Cabomba* egy másik hatalmas előnye lehet, hogy jóval nagyobb relatív növekedési ráta (RGR) értékeket produkál az árnyékosabb területeken. Ez azért lehet lényeges, mert előreláthatóan a globális klímaváltozás hatására a vizeink hőmérséklete (2-4 °C-kal (PACHAURI et al. 2014)) és tápanyag-koncentrációja (VELTHUIS et al. 2018, MEERHOFF et al. 2022) is emelkedni fog. Ezek egymás hatásait felerősítve fokozzák az úszó növényzet térhódítását (NETTEN et al. 2010). Az első tanulmányban kimutattuk, hogy a magas tápanyag-koncentráció vagy úszónövény dominanciát vagy pedig a planktonikus és a perifitikus algák dominanciáját eredményezi, aminek köszönhetően csökken a vízfelszín alatti fényintenzitás. Emiatt az árnyéktűrő képesség kulcstényezőnek bizonyulhat a *Cabomba* inváziós sikerét tekintve. Egy másik fontos tényező, amely szintén előnyhöz juttatja a *Cabombát* a *Myriophyllum*-mal szemben, hogy több mint háromszoros a nitrogénfelvétele. Azonban fontosnak tartom figyelembe venni, hogy ez a tanulmány jelentősen különbözik a természetes körülményektől és azok komplexitásától. Az eredmények laboratóriumi kísérletből származnak, így számos olyan tényező lehet, amely befolyásolhatja a különböző növénycsoportok inváziós sikerét. Ugyanakkor az eredményeink alátámasztják azokat a megfigyeléseket, amelyeket a *Cabomba* inváziójával kapcsolatosan napjainkig dokumentáltak.

A felszíni vizekben megjelenő növényi invázió nagymértékben veszélyezteti a vizes élőhelyek biodiverzitását. Eredményeink alapján az idegenhonos vízinváziók jelentősen nagyobb biomasszát termelnek az őshonos növényekhez viszonyítva, amely összhangban áll DAWSON et al. (2010) eredményeivel. Azt feltételeztük, hogy a nagyobb növekedési rátával rendelkező idegenhonos fajok sűrű állományai nagymértékben ki lesznek téve az önárnyékoló hatásnak. Ugyanakkor ezen fajok alacsonyabb levélfelület (LA) értékekkel rendelkeztek, mint őshonos társaik. Ez arra utal, hogy vagy kisebb leveleik vannak, vagy több szeldelt levéllel rendelkeznek ezzel biztosítva nagyobb felület-térfogat arányt, amely előnyös lehet a fényhasznosítás szempontjából. Ezen túlmenően a nagyobb felület-térfogat arány az önárnyékoló hatás kiküszöbölése miatt is kedvező, hiszen a kisebb méretű és/vagy szeldelt leveleket könnyebb pozicionálni az optimális fénymegkötés érdekében (RITCHIE & OLFF 1999, POORTER & ROOZENDAL 2008). Az idegenhonos fajokra jellemző magasabb levélfelületi funkcionális gazdagság értékei azt jelzik, hogy ezek a fajok levélfelület méretei jelentősen

szélesebb tartományt fednek le, amelynek köszönhetően a jellegtér sokkal nagyobb részét töltik ki az őshonos fajokhoz viszonyítva, amely szintén hozzájárulhat a hatékonyabb fénymegkötéshez (NAEM 1998). Ezeket a megállapításokat támasztják alá az idegenhonos növényközösségekben mért alacsonyabb levélfelületi funkcionális divergencia értékei is. Specifikus levélfelület (SLA) esetében nem találtunk szignifikáns különbséget az idegenhonos és őshonos fajok vonatkozásában, ami arra enged következtetni, hogy ebben az esetben az SLA nem járult hozzá a magasabb fitomassza termeléshez. Ez a megállapítás összhangban áll GUSTAFSSON & NORKKO (2019) eredményeivel, mely szerint az SLA csupán közvetett módon befolyásolja a vízinövények primer produkcióját. A fajok levél szárazanyag tartalmát (LDMC) vizsgálva nem találtunk jelentős különbséget az őshonos és idegenhonos fajok között. A növények LDMC értéke korábbi kutatások alapján összhangban áll a növényekre jellemző palatabilitással szárazföldi (PAKEMAN 2014) és vízi környezetben (ELGER & WILLBY 2003, ELGER & LEMOINE 2005, ZHANG et al. 2019) egyaránt. A funkcionális diverzitás összehasonlításához alkalmazott két különböző módszer (global pooling, optimal pooling) eredményei közötti különbség idegenhonos fajok esetében tért el nagyobb mértékben, amiből arra következtethetünk, hogy az idegenhonos fajok jelleg eloszlásai változatosabbak, amely vélhetően a nagyobb fenotipikus plaszticitásuknak és genetikai sokféleségüknek köszönhető. Általánosságban elmondható, hogy a global pooling módszer a funkcionális gazdagság értékeket túlbecsülte, mely a jelleg értékek környezethez való alkalmazkodásával magyarázható. Ugyanakkor a funkcionális divergencia értékeket alábecsülte, míg a funkcionális egyenletességet vizsgálva nem volt különbség az alkalmazott módszerek szempontjából. Elmondható tehát, hogy a két módszer eredményei közötti különbségek nagymértékben befolyásolják az idegenhonos és őshonos fajok összehasonlítását, ezért ezek alapján erősen megkérdőjelezhetőek a csak a global pooling módszer alkalmazásán alapuló vizsgálatok eredményei.

5. ÚJ TUDOMÁNYOS EREDMÉNYEK

- Az értekezés keretében bemutatott tanulmányok alapján bizonyítást nyert, hogy az epifitikus algák jelentősen gátolják mind a szubmerz-, mind pedig az úszó növények nitrogén- és foszforfelvételét.
- Kimutattuk, hogy a békalencsék növekedésgátlásában 20%-ban az epifitikus algák voltak felelősek, 80%-ot pedig a tócsagaz (*Ceratophyllum demersum*) vízkémiai paraméterekre gyakorolt hatásai (pH növekedés, tápelem-koncentráció csökkenés) okoztak, ebből kifolyólag az algák fogyasztása által a csigák képesek destabilizálni az úszó és szubmerz növények alternatív stabil állapotait.
- Strukturális egyenletmodell segítségével teljes komplexitásában sikerült feltárnunk a szubmerz és úszó növények, továbbá az epifitikus algák és az algaevő csigák közötti gátló és serkentő kölcsönhatások erősségét, figyelembe véve a tápoldat nitrogén-koncentrációját, valamint pH értékét.
- Bizonyítást nyert, hogy az idegenhonos *Cabomba caroliniana* növekedési rátája alacsony fényintenzitáson minden esetben felülmúlta az őshonos *Myriophyllum spicatum* növekedési rátáját.
- Sikerült kimutatnunk, hogy az alacsony fényintenzitás és a magasabb hőmérséklet fokozta a *Cabomba caroliniana* specifikus levélfelületét, amely az alacsonyabb levél szárazanyag-tartalommal, valamint a nagyobb nitrogénfelvételi képességgel együtt jelentősen hozzájárulhat az idegenhonos faj inváziós sikeréhez.
- Elsőként sikerült bizonyítani, hogy az idegenhonos vízínövények inváziója nem csak a fajok sokféleségére gyakorol negatív hatást, hanem az adott közösség funkcionális diverzitására is.
- Kimutattuk, hogy az idegenhonos növények jelentősen nagyobb biomasszát termelnek, amely egyrészt olyan tényezőknek köszönhető, mint a kisebb és/vagy tagoltabb levelek, melynek következtében hatékonyabbá válik a levelek pozícionálása az optimális fotoszintézis érdekében, másrészt a különböző funkcionális diverzitás indexek (a levélfelület értékeket vizsgálva magasabb funkcionális gazdagság és egyenletesség, illetve alacsonyabb funkcionális divergencia) szintén jelentősen hozzájárulnak az idegenhonos növények magasabb biomassza-hozamához.

6. IRODALOMJEGYZÉK

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7. AZ ÉRTEKEZÉS TÉMAKÖRÉBEN KÉSZÜLT PUBLIKÁCIÓK

7.1 Publikációk lektorált folyóiratokban

KOLESZÁR, G., LUKÁCS, B. A., NAGY, P. T., SZABÓ, S. (2022a): Shade tolerance as a key trait in invasion success of submerged macrophyte *Cabomba caroliniana* over *Myriophyllum spicatum*. *Ecology and Evolution*, 12, e9306. <http://doi.org/10.1002/ece3.9306>

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MELLÉKLET



The Role of Epiphytic Algae and Grazing Snails in Stable States of Submerged and of Free-Floating Plants

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ABSTRACT

Dominant floating and submerged rootless vegetation can be regarded as alternative stable states world-wide. The competition between these two vegetation types can be strongly influenced by epiphytic algae. These algae, on the other hand, are partially controlled by grazers like snails. However, how this interaction between snails and epiphyton affects the competition between floating and submerged rootless vegetation remains rather unclear. Here, we investigate this interaction. Floating (*Lemna gibba*) and submerged rootless (*Ceratophyllum demersum*) plants were co-cultured with the presence and absence of the grazing snail *Radix labiata*. Biomass and nitrogen uptake of algae were strongly reduced in the presence of grazing snails. *Ceratophyllum*-epiphyton complex without snails

reduced N and P concentration of the medium faster and had higher pH values than with the presence of grazing snails. These changes resulted in more unfavourable conditions for free-floating plants. The presence of snails indirectly increased the growth, tissue N concentration and N uptake for both *Lemna* and *Ceratophyllum*. Submerged plants together with epiphyton caused 20% more growth limitation on *Lemna* than *Ceratophyllum* alone. Structural equations modelling together with experimental results revealed that grazing snails seem to weaken the negative impact of macrophyte-epiphyton complex on *Lemna*. Large-scale field observations showed that the abundance of *L. gibba* negatively correlated with *Ceratophyllum* cover. Abundance of *C. demersum* and *L. gibba* negatively correlated with algal biomass; however, correlated positively with the group of larger sized grazing snails. Our findings strengthen the hypothesis that under a certain nutrient range, epiphytic algae stabilize the submerged vegetated state preventing colonization of lentic ponds by free-floating plants.

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Key words: Algae; *Ceratophyllum*; Epiphyton; *Lemna*; Macrophytes; Nutrients; Top-down control.

HIGHLIGHTS

- Macrophytes reduced nutrients much faster in absence of snails.
- Snails increased the growth of both floating and submerged plants.
- Epiphyton potentially inhibits the dominance of floating plants.

INTRODUCTION

In freshwater ecosystems, several well-known alternative stable states exist between primary producers. High nitrogen and phosphorus concentrations in open water bodies may alter clear water with dominance of submerged macrophytes into a turbid state with phytoplankton dominance (Scheffer and others 1993), whereas in small lentic waters this may lead towards a shift in dominance of free-floating vegetation (Scheffer and others 2003; Smith 2014). A thick layer of free-floating plants completely shades the water body, preventing the development of both submerged plants (Morris and others 2003, 2004) and algae (Pinto and O'Farrell 2014). It is also well known that shallow water combined with lower nutrient concentration may favour the growth of rooted submerged macrophytes resulting in ditches and ponds totally covered by these macrophytes (van Zuidam and Peeters 2013; Smith 2014). Beside rooted submerged plants, non-rooted submerged vegetation may also play a crucial role in the function of freshwater ecosystems. Under hypertrophic nutrient supply, coontail (*Ceratophyllum demersum*) dominated stands often form a thick layer below the surface in oxbow lakes, small ponds and ditches (Lombardo and Cooke 2003). It has been pointed out that under a certain nutrient range, non-rooted submerged vegetation is able to sustain stable dominance against phytoplankton (Dai and others 2014) and free-floating plants (Szabó and others 2021). Furthermore, it can be expected that epiphytic algae together with their grazers may significantly influence the competitive outcome between these two plant groups.

Epiphytic algae are important components in the littoral zones (Jeppesen and others 1998; Pieczynska and others 1999) because they play a key role in affecting nutrient availability together with light conditions of macrophytes (Tóth 2013; Levi and others 2015; Phillips and others 2016). Their growth is affected by different abiotic factors, such as light (Romo and Galanti 1998; Cao and others

2017), temperature (Cao and others 2017; Kanzanjian and others 2018), and nutrients (Song and others 2015; Yang and others 2020). Biotic factors such as competition for resources (Hansson 1988; Havens and others 1996; Jones and others 2002) and herbivory (Carpenter and Lodge 1986; Loman 2001) also strongly shape their abundance.

Snails are common herbivorous invertebrates in many aquatic ecosystems (Soszka 1975; Lodge 1985; Pieczynska and others 1999). Interestingly, one of the most common submerged species, *C. demersum*, was the least preferred by herbivores (Sheldon 1987; Pinowska 2002). It has been also pointed out that macrophytes are a less important part of a snail's diet, because they are mainly detritivorous and microphagous (Reavell 1980; Brown and others 1991). Instead, snails are well known to graze extensively on epiphyton attached to macrophyte tissue (Pip and Stewart 1976). Due to epiphyton removal, snails increase light availability for the plant and thus may stimulate macrophyte growth (Brönmark 1994; Bayley and others 2007; Ye and others 2019). Beside the enhanced light availability, nutrients released by snails can also stimulate the growth of macrophytes (Lodge 1991; Pinowska 2002).

Consequently, epiphyton and grazing snails together with the abiotic factors (light, nutrient concentration, temperature) may largely modify the interactions between non-rooted submerged macrophytes and free-floating plants. It has been well investigated that due to nutrient limitation, submerged macrophytes sustain their stable dominance against free-floating plants (Szabó and others 2010, 2021). However, to date there are no studies addressing the role of epiphytic algae when combined with grazing snails in this interaction.

The aim of the present study is to estimate the role of epiphytic algae in the interaction between a non-rooted submerged plant coontail (*C. demersum*) and a free-floating plant duckweed (*L. gibba*). Earlier studies showed that algae may strongly contribute to the nutrient removal from the water phase due to their faster nutrient uptake and growth rate (Roijackers and other 2004). Other studies (Song and others 2015, 2017; Yang and others 2020) indicated that under high nutrient concentrations much more epiphyton biomass was on the surface of the macrophytes because the excess of nutrients was available for the algae. It has been also pointed out that at lower concentration range, submerged vegetation strongly reduced concentration of nutrients in the water phase (Szabó and others 2010, 2021). Therefore, we hypothesized that (1) grazing snails will reduce

the biomass of the epiphytic algae and thereby lower the nutrient uptake from the water phase resulting in higher nutrient levels (2) that ultimately would lead to higher growth rate of free-floating plants. We also hypothesized (3) that the role of epiphyton in nutrient removal and in growth reduction of free-floating plants is stronger at higher than at lower concentration range where they are exposed to a stronger competition for nutrients by the submerged plants.

MATERIALS AND METHOD

Plant Collection, Preincubation

Macrophytes (*L. gibba* and *C. demersum*) and pond snails (*Radix labiata*) were collected in the middle of August from the canal Igrice, near Nyíregyháza, Hungary. The plants were cultivated on a general purpose culture solution medium detailed by Barko and Smart (1985). Nitrogen and phosphorus were supplied by adding stock solution of NaNO_3 and K_2HPO_4 to a final concentration of $5 \text{ mg L}^{-1} \text{ N}$ and $1 \text{ mg L}^{-1} \text{ P}$, respectively. The supply of micronutrients was ensured by adding 0.1 mL L^{-1} TROPICA Supplier micronutrient solution (Szabó and others 2021). The organisms were cultivated for 14 days in 20 L containers under the following conditions: $220 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photon flux density, 16:8 h light:dark, 25°C . In experimental conditions they were grown in 2 L plastic aquaria which were covered by black foil, thus light could not penetrate from the sides.

Effects of Algal Grazing on *Lemna-Ceratophyllum* Interaction

Planktic and epiphytic algal suspensions were collected from preincubated macrophyte cultures. During algal inoculation, we added 10 mL L^{-1} algal suspension to the medium. Portions of initial *Lemna* biomass (100 mg) were placed in a PVC tube as a duckweed enclosure. Overcrowding of the fronds was avoided in such a way that when *Lemna* fronds reached a 100% cover, a second series of enclosures, with a bigger diameter (respectively, 6, 7.5, 9 and 10 cm in diameter), was placed around the previous one (Szabó and others 2003). Two types of control cultures were used: algal free and algal treated (Figure 1). In *Lemna-Ceratophyllum* co-cultures, 0 g (control) and 10 g (fresh weight) *Ceratophyllum* shoots were placed outside the enclosures. Subsamples of macrophytes were taken for chemical analyses. Both in algal treated *Lemna* cultures and in *Lemna-Ceratophyllum* co-cultures, epiphytic

algal biomass was minimised by using the pond snail (*R. labiata*) as a natural algal grazer. Therefore, in one half of algal treated *Lemna* cultures and *Lemna-Ceratophyllum* co-cultures three snail individuals (1.5–3 g FW each) were placed outside the enclosures (Figure 1). To avoid consumption of *Lemna* fronds, we ensured that the snails could not enter into the *Lemna* enclosures by using transparent adhesive tape on the top of the enclosures. We used adhesive tape also at the edge of all aquaria to avoid any escaping of snails. The cultures were cultivated at low and high nitrogen concentration ($0.5; 5 \text{ mg L}^{-1} \text{ N}$, NaNO_3) with constant initial phosphorus concentration ($1 \text{ mg L}^{-1} \text{ P}$ K_2HPO_4) in triplicate. The initial pH of the medium was adjusted to 7.3. Fresh weight (FW) of *Lemna* was measured at the 4, 8 and 12 day to calculate their relative growth rates (RGR) between day 4 and 12 as $\text{RGR}_{4-12} = (\ln\text{FW}_{12} - \ln\text{FW}_4)/8$ in which FW_{12} and FW_4 were the fresh weights at time 12 and time 4, respectively.

Chemical composition of the water was followed in time in *Ceratophyllum-Lemna* co-cultures growing at $5 \text{ mg L}^{-1} \text{ N}$. Water samples were taken, measured for pH and filtered with pore size of $0.45 \mu\text{m}$ and analysed for $\text{PO}_4^{3-} \text{-P}$ and for dissolved inorganic nitrogen ($\text{NO}_3^- \text{-N}$, $\text{NH}_4^+ \text{-N}$) based on Hungarian standard methods (MSZ ISO 7150-1:1992; MSZ EN ISO 6878 2004; MSZ 1484-13 2009). At the 12 day, wet and dry weight of the plants was measured. At the end of the experiment, algae were gently removed from the surface of *Ceratophyllum* shoots and from the side of the aquaria into the media using a paintbrush. After filtration (pore diameter 5–8 μm) of the medium, total algal biomass was dried (80°C for 48 h) and dry mass was measured. Total nitrogen concentration of algae and of macrophytes growing at $5 \text{ mg L}^{-1} \text{ N}$ was analysed by dry combustion using a Vario Max Cube elemental analyzer (Elementar GMBH, Germany). Nitrogen uptake (mg) of the macrophytes was calculated from the difference between their N content in the initial subsamples and in the final biomass. Algal N uptake was calculated from the N content of final algal biomass.

Analysis of Data from Field Surveys

Using the Hungarian Biotic Database of General Directorate of Water Management (OVF, www.ovf.hu/en), we collected data (macrophyte abundance, phytoplankton chlorophyll-a, snail density) from water bodies where *C. demersum* or *L. gibba* occurred between Jun and August 2018–2019. We also used macrophyte abundance data from the

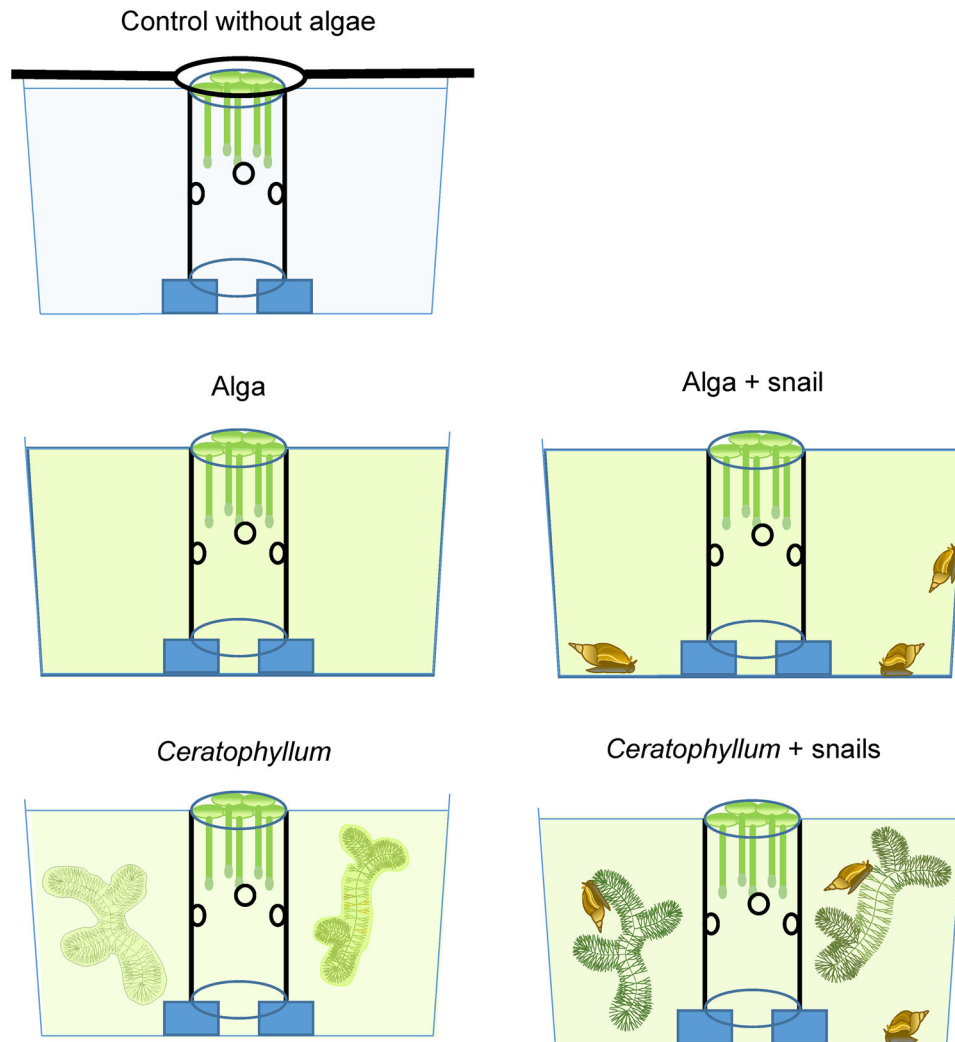


Figure 1. Experimental setup of investigating the interactions among macrophytes, algae and grazing snails.

Hungarian Biotic Database (2005–2019, $n = 296$), and from 349 surveys from 17 European countries (except from Hungary) between 1994 and 2011, including the records of either (or both) *Lemna gibba* or *Ceratophyllum demersum* (Birk and Willby 2010). Macrophyte abundance data (1–5) were converted into mean values of Braun-Blanquet's cover classes (3%; 15%; 37.5%; 62.5%; 87.5%) using the method of Engloner (2012). We also involved vegetation cover data of the two macrophytes from Dutch ditches vegetation (van Zuidam and Peeters 2013) where 70 datapoints were gathered in 2007. Vegetation recordings were done according to the Tansley coverage classes (Tansley 1946). We related the phytoplankton chlorophyll-*a* of the water and grazing snail density (number of individuals m^{-2}) with the cover of *C. demersum*. Of the 54 occurring snail taxa only large sized species (*Lymnea*, *Radix*, *Planorbis* and *Viviparus*) were con-

sidered. We also related the phytoplankton chlorophyll-*a* with the cover of *L. gibba*. To avoid disturbance by dominance of other plants if *Ceratophyllum* cover was less than 87.5%, we selected only those sites where total cover of other plants did not exceed the cover of *C. demersum* or where their total cover was less than 20%. The same filtering method was applied for the selection of *L. gibba*.

Finally, we related the cover of *L. gibba* with the cover of *C. demersum* from Hungarian field data united with Dutch ditches vegetation and data surveys of 17 European countries (Birk and Willby 2010; Szabó and others 2021).

Structural Equations Modelling

Structural equations modelling (SEM) is a multivariate statistical method which allows testing

hypotheses concerning the causal structure believed based on the observed data (Preston and others 2012). Beyond analysing the direct effect, SEM is also able to test indirect effects between two variables mediated by another intermediary variable (Bollen 1989). To improve the interpretation of differences in response variables between experimental treatments, we generated three sets of SEMs (Preston and others 2012). For *Lemna* biomass and for pH, we created a full SEM. However, since not all dependent variables (algal biomass, water N and P) were measured for the entire experiment, and because *Ceratophyllum* was applied as both independent and dependent variable, we generated two sets of restricted SEMs: one for *Ceratophyllum* and for algae from that part of the dataset where *Ceratophyllum* was present; the other for water nutrients (PO_4^{3-} -P, inorganic N) from that part of the data where *Ceratophyllum* was present at 5 mg N L^{-1} . SEM was carried out using the R package “lavaan” (Rosseel 2011; Shipley 2000). Among SEM parameters, standardized path coefficients (SPC) between two variables represent the relative strength of a relationship. We used the SPCs of full SEM for *Lemna* biomass and for pH, while we applied SPCs of restricted SEMs for *Ceratophyllum* and algal biomass and for water nutrients (PO_4^{3-} -P, inorganic N).

Statistical Methods

A general linear model (GLM) was used to test the significance of the factors (nitrogen concentration, *Ceratophyllum*, *R. labiata*) and their interactions on the variables (RGR of *Lemna*, pH of the water, RGR of *Ceratophyllum*, algal biomass,). We checked residuals for normality, and we evaluated homogeneity of variances by Levene’s test. Among *Lemna* biomass (FW_{12}) and RGR_{4-12} data, we applied Tukey post-hoc tests to evaluate which treatments differed significantly from each other. We used Pairwise Comparisons (PC) to test the variables (*Lemna* biomass, *Lemna* RGR, *Ceratophyllum* RGR, algal dry mass, pH of the water N and P removal, N uptake) for significant differences among snail treatments, where the mean difference (MD) \pm standard error (SE) was indicated.

Regarding to the field survey, the number of sites with low (0–30%) or high (> 30%) macrophyte (*C. demersum* or *L. gibba*) cover and low ($2\text{--}25 \mu\text{g L}^{-1}$) or high (> $25 \mu\text{g L}^{-1}$) algal chlorophyll-a concentration was determined. Thereafter, a Fisher’s Exact Test was applied to examine the significance of the relationship between macrophytes (*C. demersum* $n = 237$, *L. gibba* $n = 154$) and algal

chlorophyll concentration. The number of sites with low (0–30%) or high (> 30%) cover for both plant species were also determined. Thereafter, a Fisher’s Exact Test was applied to examine the significance of the relationship between *C. demersum* and *L. gibba* ($n = 715$). A regression analysis with *C. demersum* density as independent and snail density as dependent variable was performed ($n = 71$). All analyses were done in SPSS 16.0 software.

RESULTS

Effects of Grazing on Plant Growth

Nitrogen concentration of media, *Ceratophyllum* and the presence of snails had a significant ($P = 0.001$, $P < 0.001$, $P = 0.001$ ANOVA, respectively) effect on the growth rate of *Lemna* (ESM Table 1). With higher nitrogen concentration, the biomass of *Lemna* showed a significant ($P < 0.001$, MD 1.35 ± 0.121 , PC) increase (Figure 2), whereas there was no effect of nitrogen on *Ceratophyllum* growth ($P = 0.277$). Both algae ($P = 0.014$, MD 0.089 ± 0.29 , PC) and *Ceratophyllum* ($P < 0.001$, MD 0.103 ± 0.013 , PC) significantly lowered the RGR_{4-12} of *Lemna* (ESM Fig. 1).

The biomass of *Lemna* significantly ($P < 0.001$, MD 1.811 ± 0.374 , PC) decreased in the presence of *C. demersum* (Figure 2). In *Ceratophyllum-Lemna* co-cultures at low nitrogen concentration (0.5 mg L^{-1} N) there was no significant difference in the RGR of *Lemna* with and without the grazing snails. However, at high nitrogen concentration (5 mg L^{-1} N) RGR of *Lemna* was significantly higher ($P < 0.0001$, MD 0.088 ± 0.007 , PC) with than without snails (ESM Figure 1).

The RGR of *C. demersum* (day^{-1}) was also significantly ($P = 0.0144$, MD 0.01 ± 0.003 , PC) higher with snails than without them (Figure 3, ESM Table 1).

In *Lemna-Ceratophyllum* cultures, the presence of snails leads to a reduction in algal biomass by 67–86% (Figure 4A) and the dry weight of algae was significantly ($P < 0.001$, MD 90.57 ± 11.12 , PC) lower with snails than without them. At higher nitrogen concentration algal biomass was significantly ($P = 0.011$, MD 36.6 , \pm SE 11.1 , PC) higher (Figure 4A, ESM Table 1).

Effects of grazing on water chemistry and N uptake

In *Lemna-Ceratophyllum* co-cultures average pH of the medium (at day 4, 8 and 12) was significantly

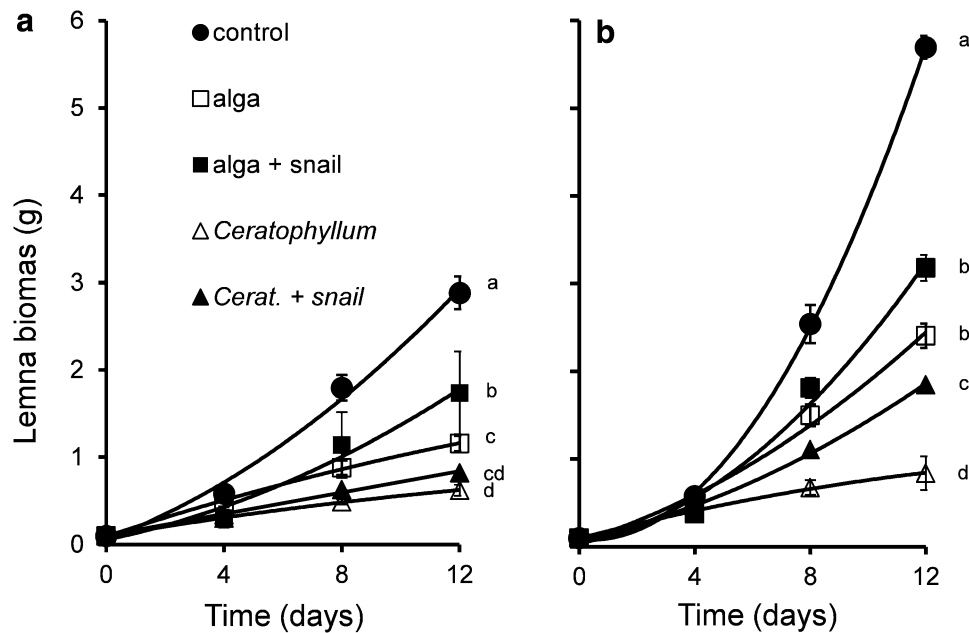


Figure 2. The impact of algae, algae with pond snail (*Radix labiata*), *Ceratophyllum* and *Ceratophyllum* with *R. labiata* on the biomass of *Lemna* cultures grown on media containing 0.5 mg L⁻¹ N (A) and 5 mg L⁻¹ N (B) (means ± SE, *n* = 3). Significant differences (Tukey's test, *P* < 0.05) of the fresh weight (day 12) among treatments are indicated with different lowercase letters.

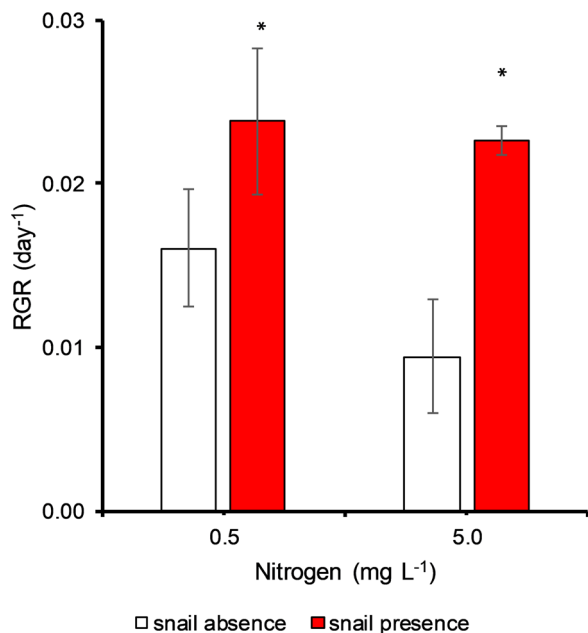


Figure 3. The relative growth rate (RGR) of *Ceratophyllum demersum* in the presence and absence of pond snail (*R. labiata*) cultivated on 0.5 and 5 mg L⁻¹ nitrogen (N) culture media (means ± SE, *n* = 3). Asterisks indicate significant differences (*P* = 0.014, PC) between snail treatments. The effect of nitrogen was not significant (*P* = 0.299, PC).

(*P* = 0.001, MD 0.378 ± 0.079, PC) lower (Figure 4B) while dissolved inorganic nitrogen (NO₃⁻-N, NH₄⁺-N) (*P* = 0.001, MD 2.70 ± 2.66, PC) and PO₄³⁻-P of the water at day 6 (*P* < 0.001, MD 0.400 ± 0.036, PC) were significantly higher with snails than without them (Figure 5A, B, ESM Table 2). In the treatment with high nitrogen concentration (5 mg L⁻¹), nutrient (N, P) removal efficiency of *Ceratophyllum-Lemna* co-cultures was mostly two times lower with than without snails during the first six days (ESM Table 2). At the end of the experiment, however, there were no significant differences in nutrient (N, P) concentrations between snail treatments. In *Lemna-Ceratophyllum* co-cultures under high nitrogen concentration, the presence of snails significantly increased the tissue N concentration and the N uptake of both macrophytes (ESM Table 2). With the presence of snails, tissue N concentration of *Lemna* and *Ceratophyllum* increased by 8 and 15%; N uptake of *Lemna* and *Ceratophyllum* increased by 128 and 194%, respectively, however, algal N uptake was lowered by 96% (Figure 5C).

Field Survey

Analysing the Database of Hungarian Surface Waters, Fisher's Exact Test showed that there was no significant relationship between *C. demersum* cover

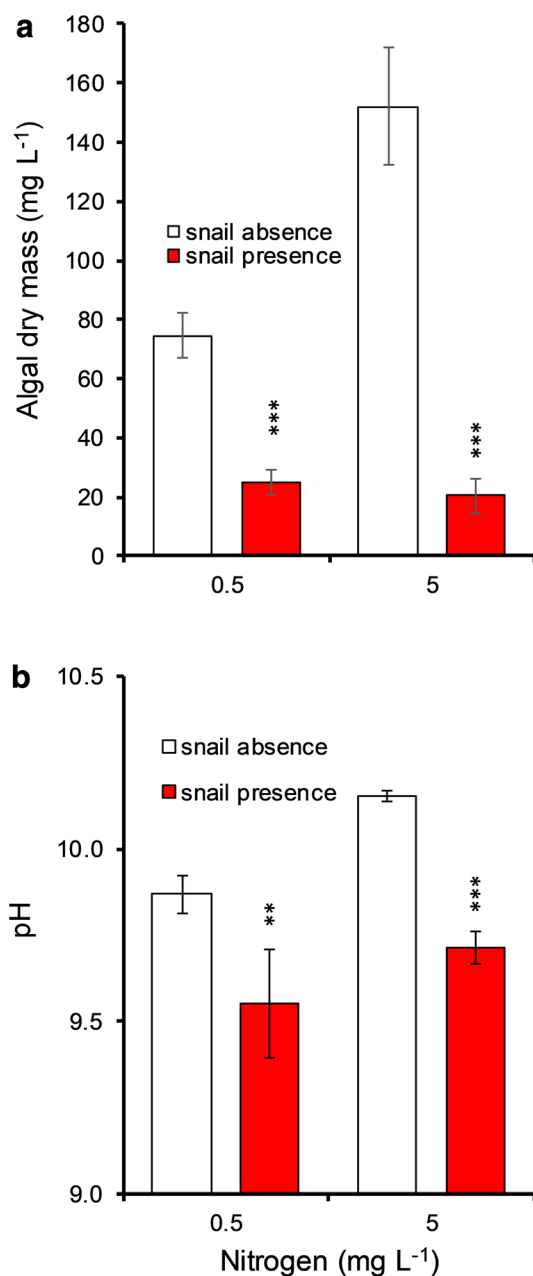


Figure 4. Total algal biomass (**A**) and average pH (day 4, 8, 12) of the water (**B**) in *Lemna-Ceratophyllum* co-cultures with the absence and presence of grazing snail (*R. labiata*) cultivated at 0.5 and 5 mg L⁻¹ N, (means \pm SE, $n = 3$). Asterisks indicate significant differences (**A** *** $P < 0.001$; **B** ** $P = 0.003$, *** $P = 0.001$ PC) between snail treatments. The effect of nitrogen was significant (**A** $P = 0.011$; **B** $P < 0.001$ PC).

and algal chlorophyll-a (Chi-Square = 0.649, $d.f. = 1$, $P = 0.540$) and between *L. gibba* cover and algal chlorophyll-a (Chi-Square = 1.174, $d.f. = 1$, $P = 0.426$). However, high phytoplankton chlorophyll only occurred at low abundances of both *C.*

demersum and *L. gibba*. As soon as phytoplankton chlorophyll-a was higher than 30 $\mu\text{g L}^{-1}$, the cover of both *C. demersum* and *L. gibba* was reduced to a very low level ($< 3\%$) (Figure 6A, B; ESM Fig. 2A, B). On the other hand, the density of grazing snails (*Lymnea*, *Radix*, *Planorbis* and *Viviparus* species) was positively correlated with the cover of *C. demersum* and this relationship was significant (ANOVA $F_{1,69} = 26.159$, $P = 0.000$) (Figure 6C; ESM Fig. 2C). Based on the European vegetation survey ($n = 715$) the Fisher's Exact Test showed that there was a significant relationship between the cover of *C. demersum* and *L. gibba* (Chi-Square = 13.090, $d.f. = 1$, $P = 0.000$). We found negative power function relationship between the cover of *L. gibba* and of *C. demersum* (Figure 6D; ESM Fig. 2D). The number of cases with high *Lemna* density (cover $> 30\%$) was 4.5 times higher in those sites where *Ceratophyllum* density was low (cover 0–30%) comparing with high *Ceratophyllum* density sites (cover $> 30\%$).

Structural Equations Modelling

Based on the standardized path coefficients computed by the full and restricted SEMs, nitrogen increased the growth both of *Lemna* (0.59) and epiphytic algae (0.33) on the other hand increasing of N from 0.5 to 5 mg L⁻¹ lowered the growth of *Ceratophyllum* (-0.26). Epiphytic algae increased the pH (+0.38) while sharply dropped inorganic nitrogen (-0.93) and phosphorus (-0.96) and lowered (-0.67) the biomass of *Lemna*. Snails strongly reduced algal biomass (-0.82) and lowered the pH (-0.52); however, they had a strong positive influence on the growth of both *Ceratophyllum* (0.71) and *Lemna* (0.42). Both *Ceratophyllum* and epiphytic algae increased the pH (0.80, 0.38) on the other hand they strongly lowered (-0.48 , -0.67) *Lemna* growth (Figure 7). Descriptive statistics of the SEMs are in ESM Table 3-5.

DISCUSSION

This was the first study to reveal the role of epiphytic algae and herbivorous snails in the complex interaction between submerged and free-floating plants. Snails strongly controlled the biomass of epiphytic algae by grazing even under hypertrophic conditions, whereas absence of snails resulted in seven times increased algal biomass with increasing nitrogen concentration (Figure 7). This strongly supports the well-known idea that epiphytic algae can shade submerged plants even by 90%, thereby decreasing their photosynthesis (Phillips and others

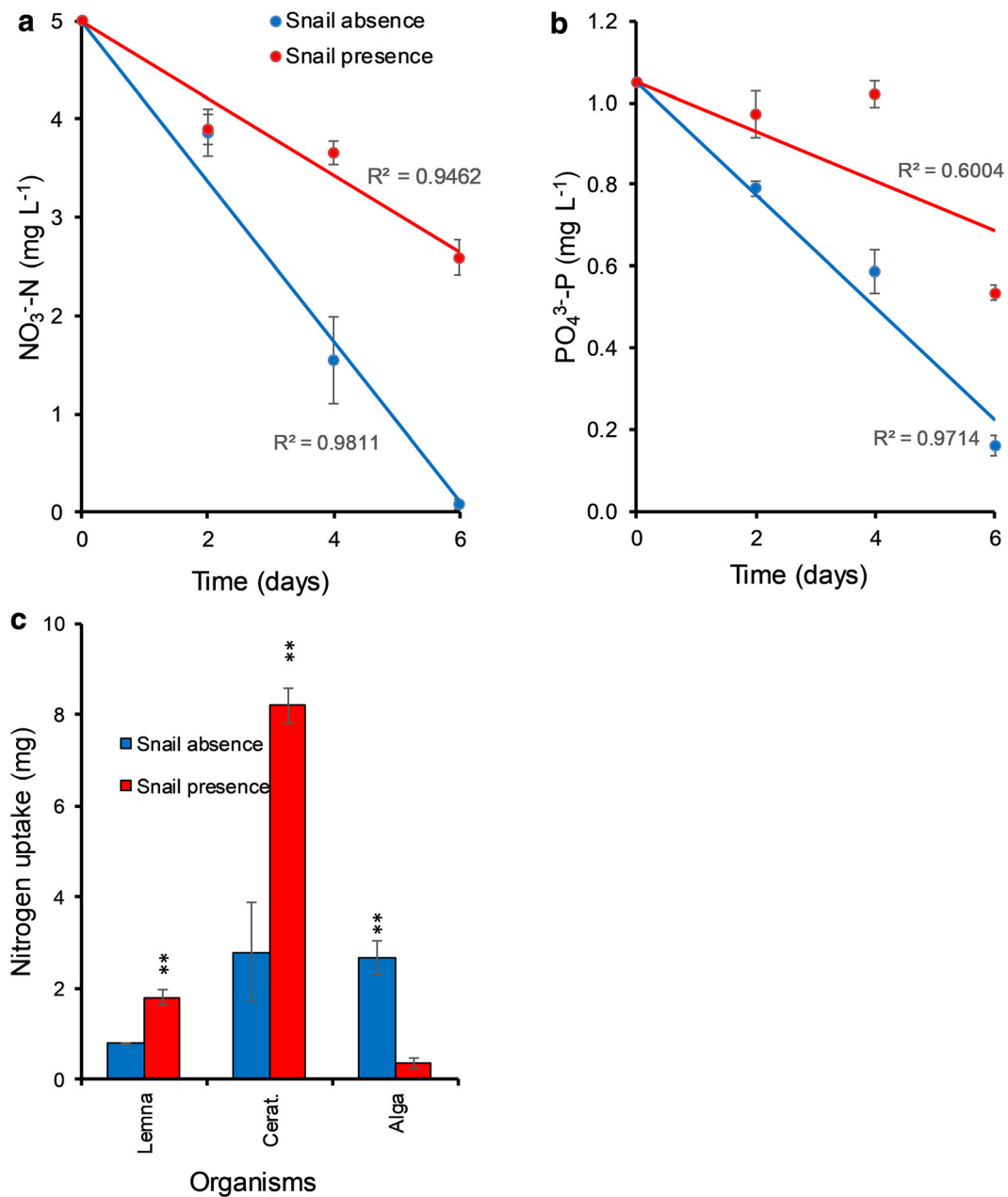


Figure 5. Nitrate (A) and phosphate (B) concentration of the water and nitrogen uptake of organisms (C) in *Lemna-Ceratophyllum* co-cultures with and without grazing snail (*R. labiata*) cultivated at $5 \text{ mg L}^{-1} \text{ N}$ (means \pm SE, $n = 3$). In panel (C), asterisks indicate significant difference between snail treatments: $P = 0.003$, 0.009 , 0.004 for *Lemna*, *Ceratophyllum* and Algae.

1978; Bulthuis and Woelkerling 1983; Tóth 2013). In addition to this shading effect, epiphytic algae form a barrier blocking nutrient uptake of submerged plants (Sand-Jensen 1977). Thus, our results are well in line with those studies that revealed the role of grazing snails in reducing the negative effect of algae on submerged plants (Cao and others 2014; Yang and others 2020).

Intense photosynthetic activity and bicarbonate uptake of macrophyte-epiphyton complex (Pedersen and others 2013) eventually leads to higher pH without than with grazing snails. In field conditions, high pH values were also detected in water bodies with high densities of submerged plants (Spencer and others 1994; Stiers and others 2011), and it was also pointed out in aquarium experiments (Roi-jackers and others 2004; Szabó and others 2005,

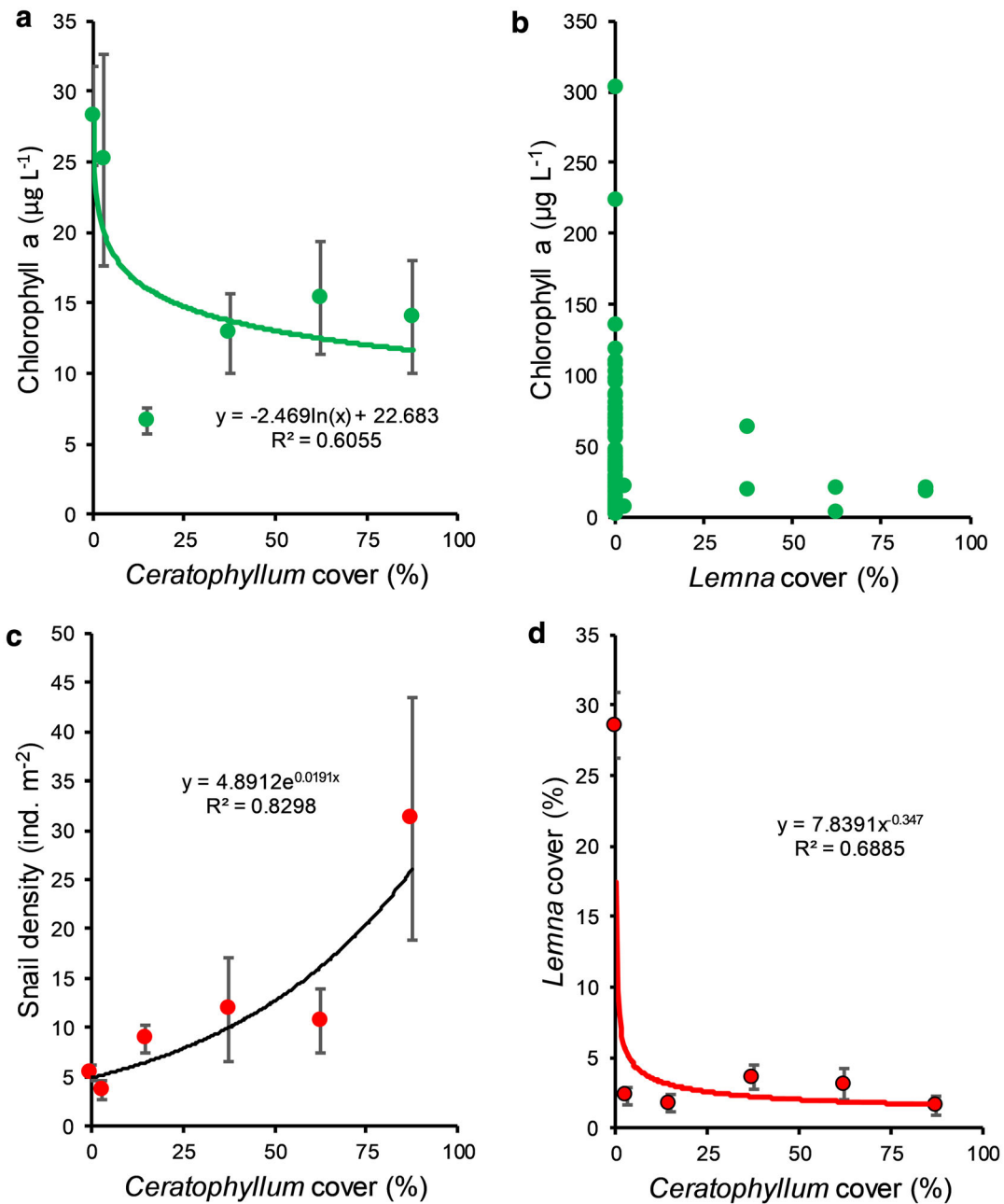


Figure 6. Correlation of phytoplankton chlorophyll-a with coverage of *Ceratophyllum demersum* (A), ($n = 237$; means \pm SE) and of *Lemna gibba* (B) ($N = 154$). Correlation of large sized snails (*Lymnea*, *Radix*, *Planorbis* and *Viviparus* species) density (C), with *C. demersum* coverage ($n = 71$; means \pm SE). Correlation of *L. gibba* with coverage of *C. demersum* (D), ($n = 715$; means \pm SE, data were $X + 0.1$ transformed).

2010). Macrophyte-epiphyton complex reduced N and P concentrations of the medium faster than snail-macrophyte-epiphyton—complex did, thereby stabilizing the submerged vegetated state resulting in more unfavourable conditions for free-floating plants. On the other hand, the resulting higher pH could be considered another stress factor for floating plants, because it strongly lowered the bioavailabil-

ity of anions (Lass and Ullrich-Eberius 1984; Ullrich-Eberius and others 1981, 1984). Because snails effectively controlled the development of epiphytic algae, contribution of the algal mat to the photosynthesis of the system has been reduced considerably. Consequently, due to top-down control of algal biomass, grazing snails indirectly reduced pH of the

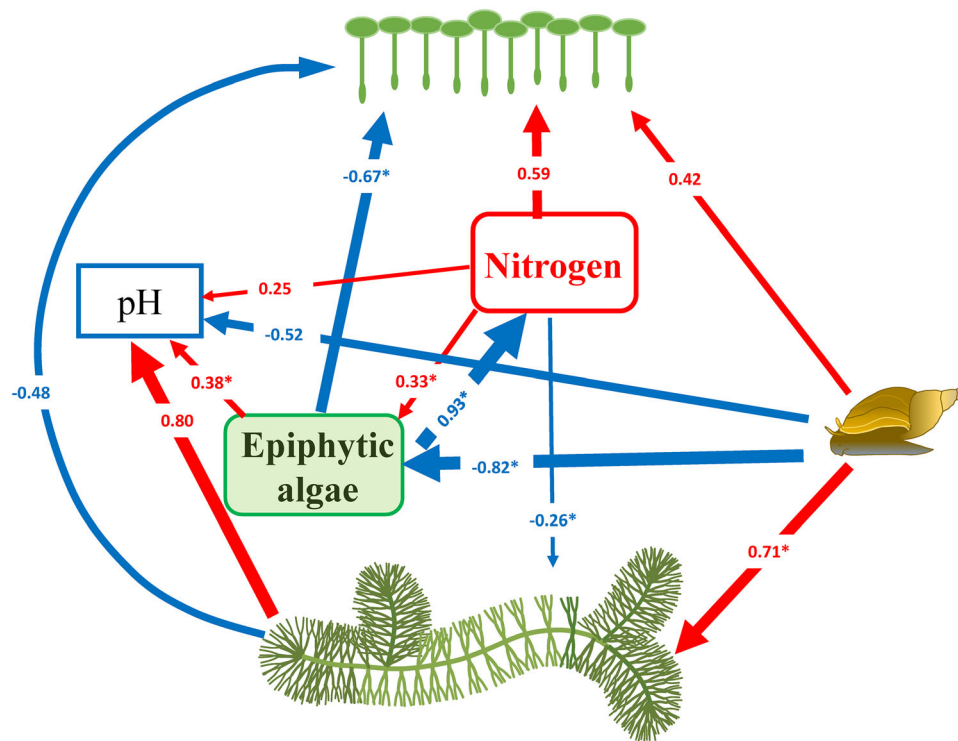


Figure 7. Interactions among abiotic factors, epiphytic algae, grazing snails, free-floating and submerged plants (*Lemna*, *Ceratophyllum*). The standardized path coefficients (SPC) computed by the full and by the restricted structural equation models (SEM) are shown on the arrows. Asterisk indicates that SPC was computed by the restricted SEMs. The relative strength of a relationship is indicated by arrow thickness (red positive, blue negative). In laboratory experiment, planktic algae contributed only 6–12% of total algal biomass, therefore are not presented.

medium resulting in a more favourable condition for free-floating plants dominance (Figure 7).

At high nutrient concentration, submerged plants together with epiphyton (absence of snails) caused 20% more growth limitation on *Lemna* than plants with epiphyton and snails. However, at lower nutrient concentration, this adverse effect of epiphytic algae on *Lemna* was insignificant since under these conditions submerged plants may already have lowered nutrient concentrations to such a level that they become potentially limiting for the algal growth (Szabó and others 2021). Based on our experimental findings, grazing of the epiphyton by the macro-invertebrate fauna can stimulate not merely the growth of submerged macrophytes as it has been well investigated (Brönmark 1985; Dudley 1992; Underwood and others 1992; Yang and others 2020), but at higher nutrient concentrations it can also indirectly increase the growth of free-floating plants.

In the complex interplay between the dominance of submerged and free-floating plants, snails have a destabilising effect on the submerged plant dominant state despite their positive effect on the plants. It has already been pointed out that with increasing

nutrients above a threshold level ($5 \text{ mg L}^{-1} \text{ N}$) submerged plant dominance weakened, while the dominance of free-floating plants strengthened (Szabó and others 2010, 2021). By consuming the epiphyton, snails reduce the negative impact (nutrient limitation, high pH) of submerged plants against free-floating macrophytes, thus allowing for free-floating plants to cover the surface completely even at lower nutrient loading.

Our field data analyses also revealed that there may be strong negative impact between planktonic algae and rootless submerged macrophytes. It seems that above an algal density threshold, the abundance of both floating and submerged plants is reduced to a low level. Since phytoplankton biomass positively correlated with epiphytic algae with increasing nutrient level (Roijsackers and others 2004) it is possible that under high chlorophyll-a concentration, the reduced submerged plant density is the result of the shading effect of the phytoplankton together with thick epiphyton cover (Phillips and others 1978, 2016). On the other hand, high submerged plant density may also cause reduced algal biomass due to competition for nutrients (Lüring and others 2006), allelopathic

substances released by non-rooted (Wium-Ander- sen and others 1983; Gross and others 2003; Dong and others 2019), or by rooted submerged macrophytes (Erhard and Gross 2006; Lürling and others 2006). In dense submerged vegetation, increased density of plankton filterers (that is, *Daphnia* sp.) may also lead to reduced algal biomass due to top-down control (Scheffer 1998). Negative correlation between planktonic algae and free-floating plants can be due to competition for light and nutrients (de Tezanos and O'Farrell 2014). Large-scale field datasets also revealed that there is a strong negative correlation between rootless submerged macrophytes (*C. demersum*) and free-floating plants (*L. gibba*). Asymmetric competition for light (high *Lemna* cover) and nutrients (high submerged plant cover) could be the background of this correlation (Scheffer and others 2003; van Gerven and others 2015; Szabó and others 2021).

In summary, different interactions exist among the investigated organisms varying in strength as indicated by the SEM (Figure 7). Submerged macrophytes are essential to epiphyton by providing substrate and organic materials. Submerged macrophytes with their epiphyton negatively impact floating plants by limiting nutrient availability in the water and increasing pH. On the other hand, shading of free-floating plants may reduce the growth of epiphyton, and as a result under intermediate density it may even stimulate the growth of submerged plants (Lu and others 2013). Above a certain threshold density, free-floating plants have an adverse effect not only on epiphyton but also on submerged plants as well (Szabó and others 2021). Snails have also a key position in this interaction network. Snails greatly benefit from macrophytes and epiphyton, which is in line with our field survey where the density of larger sized grazing snails (*Lymnaea*, *Radix*, *Planorbis*, *Viviparus* sp.) positively correlated with submerged plant (*C. demersum*) cover. Furthermore, snails stimulate the growth of submerged and, to a lesser degree, also of free-floating plants at higher nutrient concentration by consuming the epiphyton (top-down control). The ultimate impact between organisms depends on several abiotic factors like water depth, wind exposure, water movements, nutrient loading, temperature, and density of the macrophytes (Scheffer and others 2003).

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Shade tolerance as a key trait in invasion success of submerged macrophyte *Cabomba caroliniana* over *Myriophyllum spicatum*

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Abstract

The synergy between climate change, eutrophication, and biological invasion is threatening for native submerged plants in many ways. The response of submerged plants to these changes is a key factor that determines the outcome of biological invasion. In order to explain the invasion successes, we investigated the combined effects of climate change and eutrophication-related environmental factors (temperature, light, and nutrients) on the trait responses of a native (*Myriophyllum spicatum*) and an alien (*Cabomba caroliniana*) submerged species. In a factorial design, we cultivated the two species in aquaria containing low (0.5 mgNL⁻¹, 0.05 mgPL⁻¹) and high (2 mgNL⁻¹, 0.2 mgPL⁻¹) nutrient concentrations, incubated at four light intensities (average 25, 67, 230, and 295 μmol m⁻² s⁻¹ PAR photon flux density) under two temperature levels (21.5 and 27.5 ± 0.5°C). We used four invasion-related functional traits (relative growth rate (RGR), specific leaf area (SLA), leaf dry matter content (LDMC), and nitrogen to carbon ratio (N:C molar ratio)) to measure the environmental response of the species. We calculated plasticity indexes to express the trait differences between species. *Cabomba caroliniana* showed significantly higher RGR and SLA than *M. spicatum* especially under low light intensity indicating that *Cabomba* is much more shade tolerant. Elevated temperature resulted in higher SLA and reduced LDMC for *C. caroliniana* indicating that *Cabomba* may have higher invasion success. *Myriophyllum* showed higher LDMC than *C. caroliniana*. Chemical analyses of the plant tissue revealed that although *M. spicatum* showed significantly higher N:C molar ratio, nonetheless, the daily nitrogen uptake of *C. caroliniana* was more than three times faster than that of *M. spicatum*. Results supported the idea that due to its higher shade tolerance and nitrogen uptake capacity, *Cabomba* likely has greater invasion success with increasing temperature combined with low light levels.

KEYWORDS

alien, aquatic plant, competition, light, nutrient, temperature

TAXONOMY CLASSIFICATION

Biodiversity ecology, Ecosystem ecology

Koleszár and Lukács contributed equally on the study.

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1 | INTRODUCTION

The introduction of alien plant species and climate change are among some of the major global biodiversity threats. Climate change could alter almost every facet of biological invasion and every interaction between environmental stressors, thereby decreasing the resistance to invasion of natural communities (Dukes & Mooney, 1999; Hellman et al., 2007; Netten et al., 2010). Climate change is also stressing native species to the point of being unable to compete against new invasives (Rejmanek & Richardson, 1996). Moreover, in freshwaters, it intensifies the symptoms of eutrophication (Jeppesen et al., 2010), and a 2–4°C increase in water temperature is forecast by 2100 (Pachauri et al., 2014).

The synergy between climate change and eutrophication is threatening for submerged plants in many ways (Moss, 2011). On one hand, there is a positive correlation between elevated temperature and dominance of free-floating vegetation over submerged plants (Peeters et al., 2013). In addition, high nutrient concentration of the water may also result in the dominance of phytoplankton (Scheffer & van Nes, 2007) and free-floating plants (Scheffer et al., 2003; Smith, 2014; Szabó et al., 2022). Both phenomena cause light limitation (Lewis & Bender, 1961; Morris et al., 2003; Phillips et al., 2016), which negatively affects the growth of submerged plants. The response of submerged plants to these changes (i.e., lower light intensity, higher nutrient concentration, and elevated temperature) is a key factor that determines the outcome of biological invasion.

In the naturalization phase of invasion, alien species must adapt to the new environmental conditions in order to establish, survive, and reproduce (Richardson & Pyšek, 2012). Morphological or physiological characteristics (i.e., specific leaf area (SLA), dry matter content, relative growth rate (RGR), and nutrient uptake) that provide a competitive advantage to alien plants over native species have a key role in this process (Vilà & Weiner, 2004). Greater phenotypic differences between alien and native aquatic plants considerably increase the probability of the alien's success (Lake & Leishman, 2004). Accordingly, higher RGR, nutrient uptake, SLA, and higher phenotypic plasticity can contribute to the success of alien aquatic plants (Geng et al., 2006; Lukács et al., 2017; Szabó et al., 2019, 2020). Light, nutrient availability, and temperature are strongly related to climate change and eutrophication. Responding to these environmental factors, invasive plants need to be able to change their phenotypic properties more quickly, such as elongation (Molnár et al., 2015; Szabó et al., 2019), branching, root-shoot ratio (Szabó et al., 2019), chlorophyll concentration (Szabó et al., 2020), leaf area (Riis et al., 2010), or dry matter content (Larson, 2007). The responses to these factors separately have been well documented; however, there is not a single study addressed to evaluate their combined impact on morphological and physiological traits of submerged macrophytes.

Cabomba caroliniana is one of the macrophytes that gains high invasion risk in freshwaters (Matthews et al., 2017). Significant differences were discovered between *C. caroliniana* and native beds for underwater light conditions, macrophyte equitability, and epiphytic algal biomass (Hogsden et al., 2007). Roijackers (2008) stated

that growth form plays an important role in the competitive ability of *Cabomba caroliniana*. Specifically, where there are plants with a similar growth form (e.g., *Myriophyllum spicatum* and *M. heterophyllum*), the growth potential of *C. caroliniana* appears to be limited. However, limited information was found on the effects of *C. caroliniana* on native aquatic plants (Matthews et al., 2013).

In our experimental study, we compared the phenotypic plasticity of *Cabomba caroliniana* and *Myriophyllum spicatum*, two submerged aquatic plants with similar growth form (i.e., Myriophyllid: anchored submerged plants with long stems and finely divided submerged leaves, Wiegleb, 1991). *Cabomba caroliniana* (Cabombaceae) is a fast-growing submerged aquatic plant, native in Argentina, Brazil, Uruguay, Paraguay, and South-eastern USA (Ørgaard, 1991); however, it forms densely vegetated stands in European freshwaters as an alien species; frequently distributed in the UK (Stace, 1997), the Netherlands (van der Velde et al., 2002), and Hungary (Lukács et al., 2014; Steták, 2004). *Myriophyllum spicatum* (Haloragaceae) is native in Europe, Asia, and North-Africa (Patten, 1954). We have chosen to compare the environmental response of these species because they frequently occur in the same habitat in Hungary, and we suggest strong competitive interaction between them due to the same growth form display similar extent of realized niche (Begon et al., 1996).

Specific leaf area, leaf dry matter content (LDMC), nitrogen/carbon (N:C) molar ratio, and RGR are considered as the so-called “response” traits in the plant trait literature (Engelhardt, 2006). It means that it can be used to examine the manner in which biota responds to changes in the environment; therefore, we investigated these traits in order to get answers to our questions.

We hypothesized (H1) that differences in their growth rate and nutrient uptake become more pronounced both under higher temperature or higher nutrient concentration and with decreasing light conditions. We suggest that these factors may contribute to the invasion success of *Cabomba* over *Myriophyllum*. We also hypothesized (H2) that along various environmental conditions, *Cabomba* has a higher phenotypic plasticity than *Myriophyllum* and this may also contribute to its invasion success.

This study aims to evaluate these hypotheses by investigating the combined effects of temperature, light, and nutrients (N, P) on the trait responses of the two species in a laboratory experiment. A further aim is to clarify the limited competition ability of *Cabomba* growing next to *Myriophyllum*, and how this competition is altered by changing environmental conditions. Since submerged species may strongly change light conditions if they are grown in cocultures (Szabó et al., 2019), we cultivated them separately in order to eliminate these effects.

2 | METHOD

2.1 | Plant collection, preincubation

We collected apical shoots of *Cabomba caroliniana* from the thermal outflow of Lake Hévíz (N 46.786986°, E 17.194127°), and *Myriophyllum spicatum* from the Eastern Principal Channel (N

47.860911°E, 21.382270°), Hungary. Shoots were preincubated in plastic boxes containing 20 L of deionized water which was supplemented by Na^+ , K^+ , Ca^{2+} , Mg^{2+} , HCO_3^- , SO_4^{2-} , and Cl^- as general purpose culture solution medium detailed by Barko and Smart (1985). Final concentration of the solutions for nutrients varied from eutrophic (0.5 mgNL^{-1} and 0.05 mgPL^{-1}) to hypertrophic (2 mgNL^{-1} and 0.2 mgPL^{-1}) through the treatment of adding NH_4NO_3 and K_2HPO_4 stock solutions (1000 mgL^{-1} for N and for P) to the medium. The nutrient (nitrogen and phosphorus) concentration of medium was set to these values because we intend to simulate the natural conditions and these two trophic levels refers to eutrophic and hypertrophic status of natural waters. The supply of micronutrients was ensured by adding TROPICA Supplier micronutrient solution (Szabó et al., 2010). We preincubated the selected apical shoots (10–12 cm length) for 14 days under $230 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photosynthetically active radiation (PAR), 16:8 h L/D regime at $24.5 \pm 0.5^\circ\text{C}$. We renewed the culture medium twice a week. Shoot length nearly doubled (22 cm) under 14 days of preincubation. Therefore, most of the selected apical shoots (11–14 cm) had developed already under preincubation period; hence, they were well accommodated for experimental condition. Before starting the experiment, we removed water from the surface of the plants using a centrifuge (600 RPM, 10 s). We measured subsamples of initial shoots of each species from each nutrient concentration for fresh weight (FW) and dry weight (DW) (W_0).

2.2 | Laboratory experiment

We placed six apical *Cabomba* and *Myriophyllum* shoots (11–14 cm length, $7.40 \pm 0.2 \text{ g}$ FW each) separately in 2-L aquaria (height: 11.5 cm, width: 11.5 cm, length: 18 cm) containing the culture media described above. The initial pH of the water was adjusted to 7.3. Plant shoots were placed free into the aquaria, and they were not planted into a substrate. They were positioned that way to exclude the self-shading effect. We covered the sides of the aquaria with black foil to avoid light penetration from the sides. For both species, two different nutrient treatments (0.5 mgNL^{-1} , 0.05 mgPL^{-1} ; 2 mgNL^{-1} , 0.2 mgPL^{-1}) were incubated at four different light intensities varying from strongly shaded to well-illuminated conditions: 22–28 (L1), 52–82 (L2), 170–290 (L3), and 260–330 (L4) $\mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR photon flux density. The highest light intensity used in the experiment is roughly the same that we could measure on a summer sunny day under half-shaded conditions in a natural water. The other light intensities were set to this values because the differences between light treatments were suitable for measuring the effects of light intensity on the plants. The light intensity of lamps was not dimmable, but the distance between the lamps and aquaria was adjustable; therefore, light intensity could be modified in a specific area. We measured the light intensity at the water surface of each aquaria, and they were placed into a lane where the light intensity was appropriate for the treatment. The plants were grown under moderately cold and

warm water (21.5 and $27.5 \pm 0.5^\circ\text{C}$) using a controlled temperature water bath. We renewed the culture medium on days 2, 4, and 6. Illumination was carried out by 400 W metal halogen lamps. Each treatment ($2 \times 2 \times 2 \times 4 = 32$) was replicated three times meaning that we used 96 aquaria. We finished the experiment after 8 days in order to keep the initial light levels more or less constant, as well as to avoid overcrowding of the plants.

2.3 | Relative growth rate, Specific leaf area, and leaf dry matter content

Following Pérez-Harguindeguy et al. (2016) protocol, we selected three relatively young (presumably more photosynthetically active) but fully expanded and hardened leaves from the upper and middle sections of the shoots from each aquarium ($96 \times 3 = 288$ leaves). We measured the area of the leaves using a LI-3000 Leaf area meter + LI-3050C Transparent Belt Conveyor Accessory (LI-COR Biosciences GmbH, Germany). The whole submerged plants and the three cut leaves were used for fresh weight and dry weight determination. We dried samples at 80°C for 48 h. After that, we immediately measured their weights on Ohaus Adventure Pro scale. The RGR of the plants was calculated as $\text{RGR} = (\ln W_t - \ln W_0)/t$, where W_0 represents the initial and W_t the final dry weight of the three plants in each aquarium, and t is the cultivation time in days.

We applied two additional traits in the subsequent analyses; both were calculated from the measured leaf area, leaf fresh weight, and leaf dry weight data.

Specific leaf area (SLA) was calculated as $\text{SLA} = (\text{LA}/W_t \text{ mm}^2 \text{ mg}^{-1})$ where LA represents the leaf area and W_t the dry weight of the leaves. We applied SLA because it tends to scale positively with mass-based light-saturated photosynthetic rate, and in general, species tend to have higher SLA in permanently or temporarily resource-rich environments than do those in resource-poor environments.

Leaf dry matter content (LDMC) was calculated as $\text{LDMC} = (W_t/W_0 \text{ mg g}^{-1})$, where W_0 represents the initial (water saturated) and W_t the final dry weight of the leaves. Leaves with high LDMC tend to be relatively tough and are thus assumed to be more resistant to physical hazards.

2.4 | Chemical composition

At the end of the experiment, we analyzed nitrogen and carbon concentration of the dried plants (96 samples) by dry combustion using a Vario Max Cube elemental analyzer (Elementar GmbH, Germany).

2.5 | Plasticity index

We calculated the plasticity index (PI) for RGR, SLA, and LDMC for light (PI_L), nutrients (PI_N), and temperature (PI_T) according to Szabó et al. (2019) as: $\text{PI} = (\text{maximum mean} - \text{minimum mean})/\text{maximum}$

mean. The index ranges from 0 (no plasticity) to 1 (maximum plasticity).

2.6 | Statistical analysis

Normality of the variables was checked by the Kolmogorov–Smirnov test. RGR, SLA, and LDMC were all normally distributed ($p > .05$). A general linear model (GLM) was used to test the significance of the factors (light, nutrient, temperature, and species identity) and their interactions on the variables. Pairwise comparisons (PC) were used to test the variables for significant differences between species where mean difference (MD) \pm standard error was indicated; furthermore, ANOVA was used to test the significance level when examining the effect of an independent variable on a dependent variable for a given species. All analyses were made using SPSS 16.0 software.

3 | RESULTS

3.1 | Relative growth rate

For the overall experiment, all independent variables (light intensity, nutrient concentration, and temperature) have a significant effect on the RGR of both species (Table S1). Increasing temperature significantly (MD = -0.011 ± 0.005 , $p = .018$) reduced the RGR of submerged plants at low (L1 & L2) light intensity (Figure 1b, d). RGR of *M. spicatum* measured at high nutrient concentration was significantly higher (MD = 0.009 ± 0.003 , $p = .007$) than at low concentration; nevertheless, it was not affected by temperature treatments. Nutrient concentration significantly influenced ($F = 9.049$, $p = .005$) the growth of *C. caroliniana*. For the overall experiment, *C. caroliniana* showed significantly higher RGR than *M. spicatum* (MD = 0.011 ± 0.003 , $p = .001$) (Figures 1 and 5). Moreover, at lower light intensity (L1 and L2), the differences were even larger (MD = 0.022 ± 0.004 , $p < .001$). However, at high light intensity (L3 and L4), there were no significant differences detected between growth rates of plant species (Figure 1).

3.2 | Specific leaf area

For the overall experiment, light intensity, species identity, and temperature had a significant effect on the SLA, but nutrient concentration did not (Table S1). On one hand, SLA of *M. spicatum* was not altered at all neither by nutrient concentration, light intensity, or temperature. By contrast, SLA of *C. caroliniana* was affected both by light intensity ($F = 3.413$, $p = .029$) and temperature ($F = 18.464$, $p < .001$) (Figure 2). SLA of *C. caroliniana* was significantly higher at high temperature (MD = 13.1 ± 3.0 , $p < .001$). Light intensity had a significant (MD = 20.0 ± 4.1 , $p < .001$) effect on SLA of *C. caroliniana* only at low temperatures. In all cases, SLA of *C. caroliniana* was

significantly (MD = 48.5 ± 2.0 , $p < .001$) higher than that of *M. spicatum* (Figures 2 and 5).

3.3 | Leaf dry matter content

Species identity and temperature had a significant effect on LDMC (Table S1). LDMC of *M. spicatum* was significantly higher (MD = 0.061 ± 0.004 , $p < .001$) than that of *C. caroliniana* (Figures 3 and 5) in all light intensity treatments. However, the differences were not significant under low temperature ($21.5 \pm 0.5^\circ\text{C}$) combined with low light intensity (L1) (Figure 3). *Cabomba caroliniana* showed reduced LDMC value with higher temperature (MD = 0.020 ± 0.006 , $p = .002$).

3.4 | N:C molar ratio

For the overall experiment, nutrient, light, and species identity had a significant effect on the N:C molar ratio (Table S1). Overall, *M. spicatum* had a significantly higher (MD = 0.013 ± 0.001 , $p < .001$) N:C molar ratio than *C. caroliniana*; furthermore, under low light conditions (L1 and L2), the differences between species were even greater (Figures 4 and 5). On the other hand, at low light conditions (L1 and L2) with high temperature, the daily nitrogen uptake ($\text{mg N g FW}^{-1} \text{ day}^{-1}$) of *C. caroliniana* was significantly higher (MD = 0.100 ± 0.021 , $p < .001$) and 3.6 times faster than that of *M. spicatum* (Figure 6).

3.5 | Differences in phenotypic plasticity

Along the examined light gradient combined with temperature and nutrient levels, the two submerged species showed marked differences in their phenotypic plasticity (Table 1). On one hand, *M. spicatum* showed greater (by 0.1) plasticity for light regarding to RGR. On the other hand, *C. caroliniana* showed higher (by 0.1) plasticity for light and temperature in SLA and LDMC. With regard to nutrients, the two species did not show any characteristic differences in their phenotypic plasticity values. Regarding the overall phenotypic characteristics, *C. caroliniana* showed a higher plasticity than *M. spicatum*.

4 | DISCUSSION

The search for invasive traits and the investigation of trait plasticity constitutes a challenging task in freshwater biology and invasion biology. The best progress toward a general conclusion of this issue would be to pool evidence from pairwise comparisons and multi-species studies (Pysek & Richardson, 2007). In the case of aquatic plants, comparisons within growth forms have high relevance due to the scarcity of congeneric alien-native species pairs. Since growth

FIGURE 1 Relative growth rate (RGR [dry weight]) of *Myriophyllum spicatum* and *Cabomba caroliniana* cultures grown at different light levels and treatments (a) low nutrient and low temperature; (b) low nutrient, high temperature; (c) high nutrient, low temperature; (d) high nutrient and high temperature (mean \pm SE, $N = 3$). Asterisks indicate a significant difference (PC) between the species (* $p < .05$, ** $p < .01$).

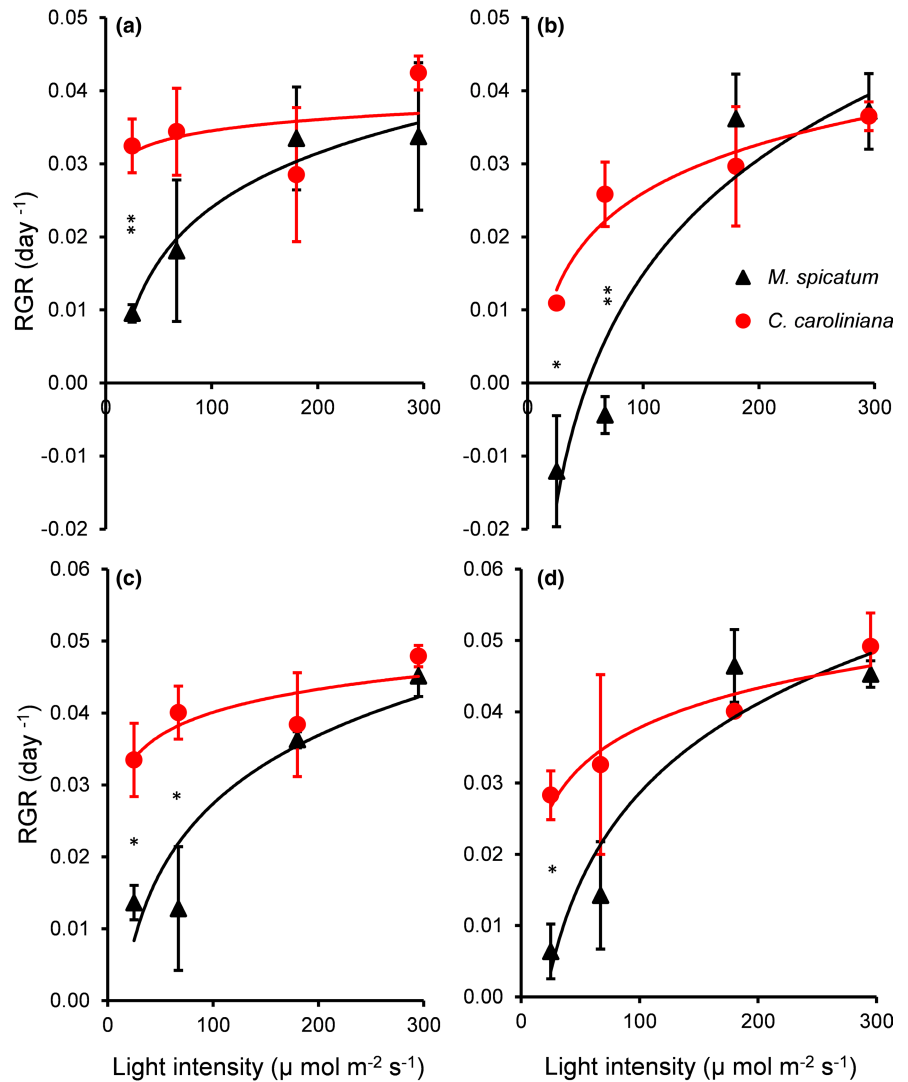
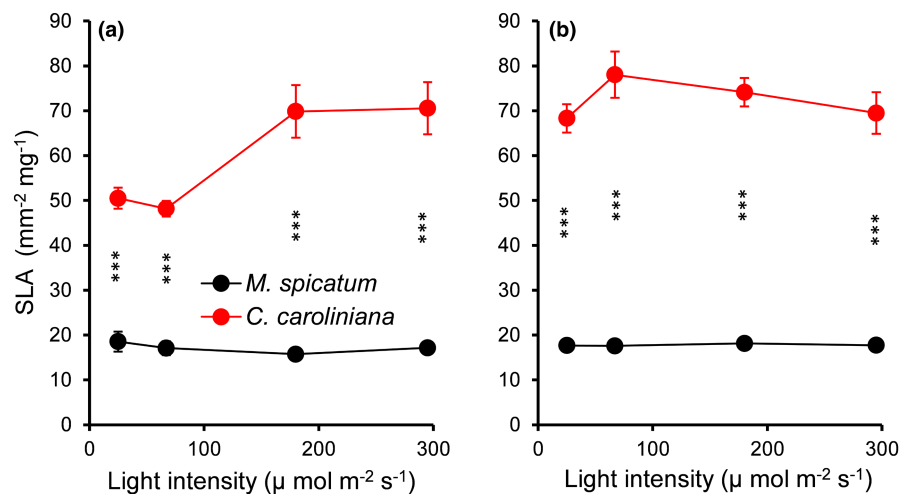


FIGURE 2 Specific leaf area (SLA $\text{mm}^{-2} \text{mg}^{-1}$) of *Myriophyllum spicatum* and *Cabomba caroliniana* cultures grown at different light levels combined with low (a) and high (b) temperature. Each point represents data from low and high nutrient concentrations (mean \pm SE, $N = 6$). Asterisks indicate a significant difference (PC) between the species (** $p < .001$).



forms are stem from certain combination of traits, it can be conceptualized as groups of plants with similar degree of adaptation (Rowe & Speck, 2005). Anchored submerged plants with long stems and finely divided submerged leaves (i.e., Myriophyllid growth form) are successful in colonizing flowing waters (Wiegleb, 1991), since finely

divided leaves give extra competitive advantages over species with entire leaves (Givnish, 1987). These advantages is due to the fact, that divided leaves have much more light capturing surface per unit biomass than entire leaves, and thereby they can be more efficient in photosynthesis. In consideration of this and its high natural

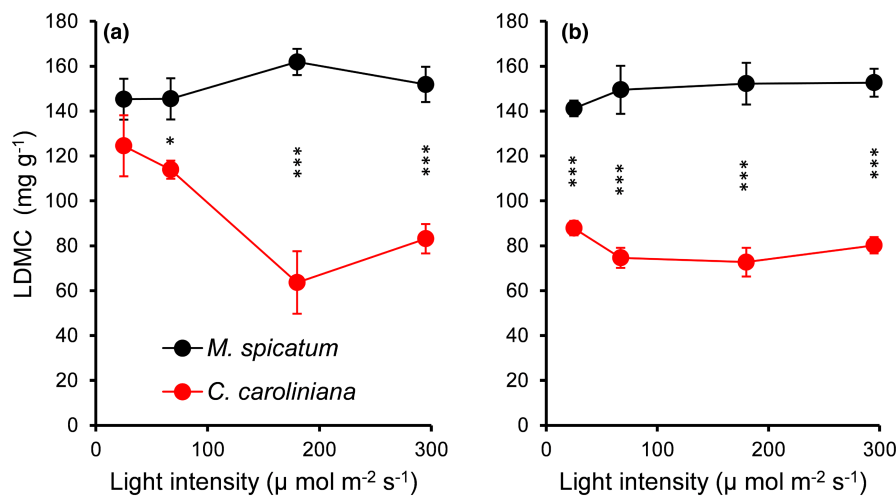


FIGURE 3 Leaf dry matter content (LDMC mg g⁻¹) of *Myriophyllum spicatum* and *Cabomba caroliniana* cultures grown at different light levels combined with low (a) and high (b) temperature. Each point represents data from low and high nutrient concentrations (mean ± SE, N = 6). Asterisks indicate a significant difference (PC) between the species (*p < .05, ***p < .001).

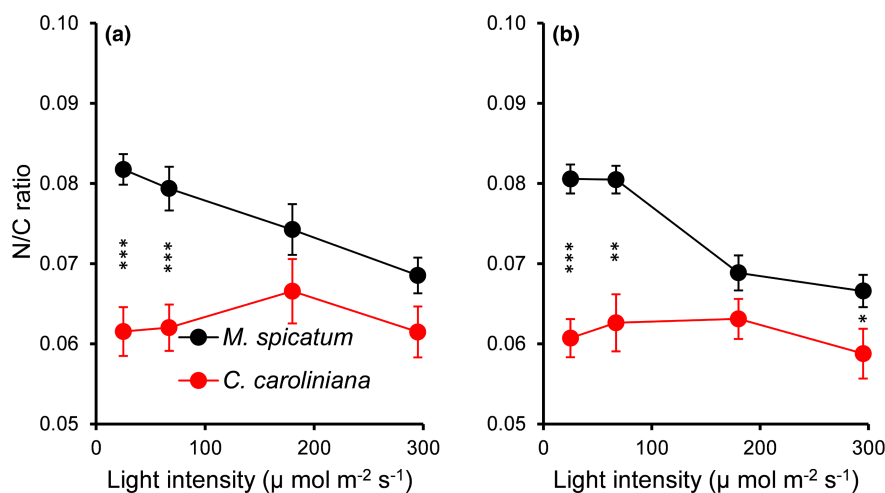


FIGURE 4 N:C molar ratio of *Myriophyllum spicatum* and *Cabomba caroliniana* cultures grown at different light levels combined with low (a) and high (b) temperature. Each point represents data from low and high nutrient concentrations (mean ± SE, N = 6). Asterisks indicate a significant difference (PC) between the species (*p < .05, **p < .01, ***p < .001).

dispersal potential, it is not surprising that *Cabomba caroliniana* is among the most successful invasive aquatic plants across Europe (Hussner, 2012).

Our laboratory results supported the idea that competitive success of the alien *C. caroliniana* comes from its higher specific leaf area (SLA) and 2–3 times higher growth rate under shaded conditions than *M. spicatum*, which indicated that *C. caroliniana* tolerated the shade better (more shade tolerant). Under shaded conditions, higher temperature induced even higher specific leaf area (SLA) for *Cabomba*. Therefore, our results are in line with the finding of Lukács et al. (2017) who pointed out that among *Myriophyllids* the invader species had higher SLA than natives. Furthermore, it also supports the findings of Lake and Leishman (2004) and Hamilton et al. (2005) who found that high SLA can promote invasiveness.

In addition, higher SLA and lower LDMC of *Cabomba* versus *Myriophyllum* suggest that *Cabomba* can form much more leaf area from the same dry matter content (LDMC), which can also contribute to its invasion success. Invaders having lower LDMC may have an advantage in the competition for light, because softer leaf tissues allow invaders to build their photosynthetic organs faster and invest less into structural tissue elements (Lukács et al., 2017). Our results support those findings revealing that some invader species can invest much more on relative shoot elongation (mm g⁻¹ DW) which

can provide a better position for light capture (Szabó et al., 2019). It also suits the finding that alien species can develop larger leaf area faster, thereby increasing their invasion success (Lukács et al., 2017).

Since former studies have already pointed out that *Cabomba* has high light requirements (Hiscock, 2003; Scheurmann, 1993), it was expected that low light intensity would have a limiting impact on its growth. However, in our experiment, *Cabomba* showed much less reduction in growth at low light levels than did *Myriophyllum*. Additionally, the growth rate of *Cabomba* was two–three times higher than that of *Myriophyllum*, indicating its lower light compensation point compared with *Myriophyllum*. Under natural conditions, especially in eutrophic waters, epiphytic algae can shade submerged plants by up to 90%, decreasing their photosynthesis and growth (Bulthuis & Woelkerling, 1983; Koleszár et al., 2021; Phillips et al., 2016; Tóth, 2013). However, both species are able to produce allelopathic substances against blue green algae, thereby partly lowering the shading effect of periphyton (Nakai et al., 1999). In this way, those species equipped with a trait such as lower light compensation point might gain extra competitive advantage. Our results indicate that *Cabomba* can be more competitive species than *Myriophyllum*, and it may survive better under more shaded eutrophic conditions, such as turbid water or below a mat of floating plants (Szabó et al., 2020; van Gerven et al., 2015).

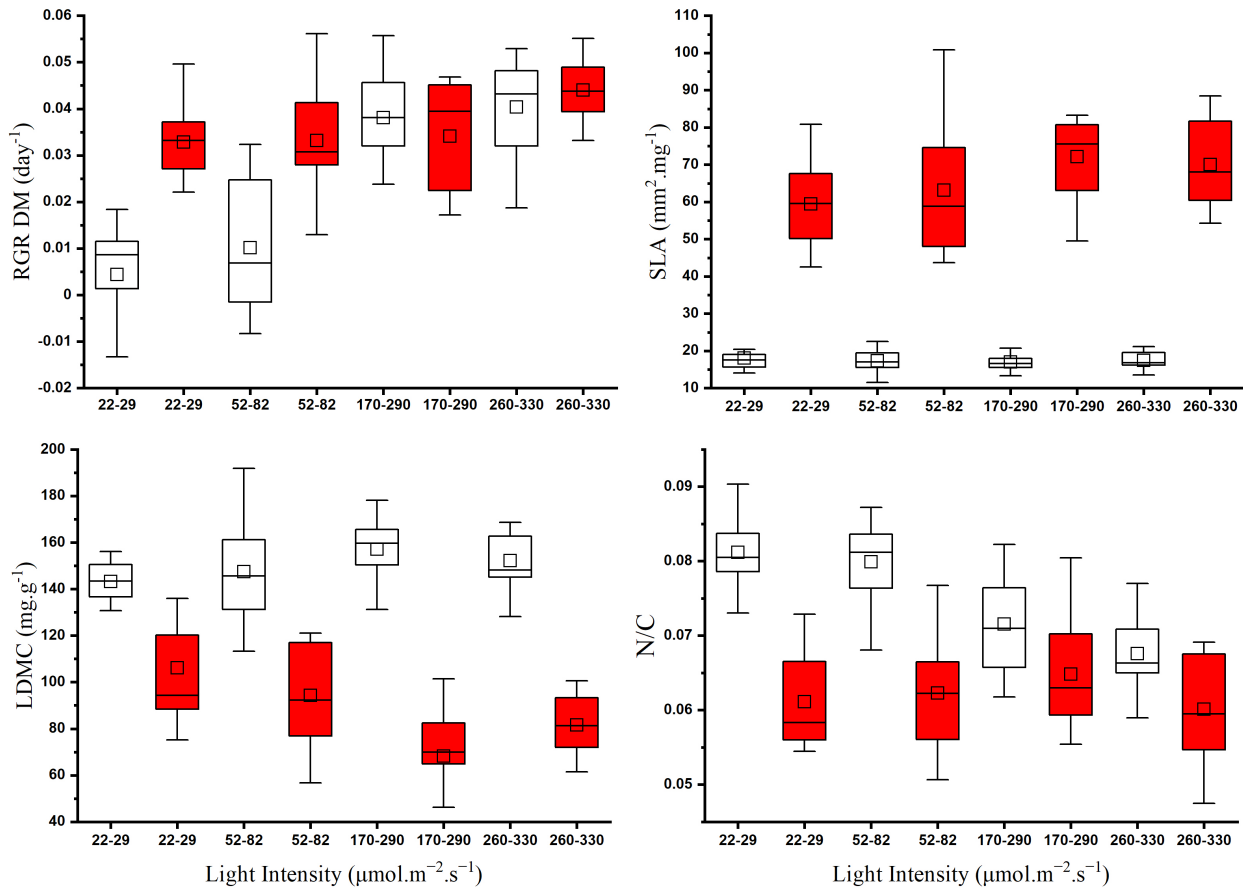


FIGURE 5 Boxplots of relative growth rate (RGR [dry weight]), specific leaf area (SLA), leaf dry matter content (LDMC), and N:C molar ratio of *Myriophyllum spicatum* (white) and *Cabomba caroliniana* (red) under different light levels. Each boxplot represents data of four light intensities. Boxes: +25%–75% percentiles; whiskers: Standard deviations, □: Median, $n = 12$.

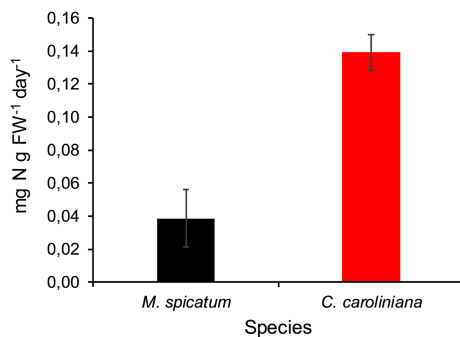


FIGURE 6 Nitrogen uptake ($\text{mg N g FW}^{-1} \text{ day}^{-1}$) of *Myriophyllum spicatum* and *Cabomba caroliniana* cultures grown at low light levels (L1 & L2) with high temperature. Each column represents data from low and high nutrient concentrations (mean \pm SE, $N = 12$).

In our study, higher nutrient concentration only slightly enhanced the RGR of *Myriophyllum*, and *Cabomba*. On the contrary, analyzing the tissue N concentration revealed that under low light intensity, *Cabomba* has more than three times higher nitrogen uptake capacity than *Myriophyllum* (Figure 6). This trait may be especially advantageous in waters with suboptimal nitrogen concentration. Furthermore, at low light intensity, increasing temperature reduced the growth rate of both submerged plants, but significantly

enhanced the SLA of *Cabomba* and reduced its LDMC likely contributing to its invasion success.

The aim of our study was to investigate the combined effects of temperature, light, and nutrients (N, P) on the trait responses (RGR, SLA, LDMC, N:C molar ratio) of the two species in an 8-day laboratory experiment. However, several questions may arise regarding the duration of the experiment. On the one hand, the plants were properly preincubated since shoot length and biomass nearly doubled in the 14 days of preincubation (via RGR 0.04 day^{-1}); thus, after cutting the apical end, newly developed shoots were used for the experiment. On the other hand, this period was sufficient to find well-detectable significant changes in the plant traits due to the various light intensities and/or nutrient concentrations and/or temperature levels, as mentioned above. Although an 8-day period experiment may seem relatively short, the reason we finished the experiment after 8 days was to avoid overcrowding of the plants in 2-L aquaria. In case of overcrowding, the changes in traits are no longer just due to the treatments (light, nutrient, and temperature), but also due to self-shading (intraspecific competition). Our results under noncrowded laboratory conditions with low light levels partly imitated those field conditions with densely growing shaded plants where the invasion is actually happening. Thus, the measured growth traits along light gradient gave relevant information for field conditions as well. Over

Variable	Myrioph.	Cabomba	Myrioph.	Cabomba	Myrioph.	Cabomba
	PI _{Light}		PI _{Nutrient}		PI _{Temperature}	
RGR	0.89	0.40	0.31	0.22	0.17	0.15
SLA	0.07	0.20	0.07	0.01	0.04	0.18
LDMC	0.06	0.29	0.05	0.05	0.01	0.21

TABLE 1 Plasticity index (PI) of *Myriophyllum spicatum* (*Myrioph.*) and *Cabomba caroliniana* (*Cabomba*) for light (PI_L), nutrients (PI_N), and temperature (PI_T). Greater PI values are bold if differed by 0.1.

and above, there are several studies (Cedergreen et al., 2004; Huang et al., 2017; Szabó et al., 2019, 2020) clearly supported that 14 days of preincubation with 8-day incubation was sufficient to compare traits (RGR, LDMC, N:C molar ratio, SLA) between aquatic plant species.

Our short-term experimental results pointed out that the three environmental factors significantly modified the investigated plant traits. However, it is well known, that these abiotic conditions constantly change over longer growing periods. Due to apical elongation of the shoots, it is obvious that light intensity increases with decreasing water depth (Pokorný et al., 1984). On the contrary, under hypertrophic conditions, shading of epiphytic algae can strongly decrease light conditions on the surface of the older leaves (Levi et al., 2015; Phillips et al., 2016; Tóth, 2013). Bioturbation of benthic fauna may not only decrease light conditions, but also increase nutrient release from the sediment to the water body as well (Adámek & Maršálek, 2013; Chen et al., 2016; Scheffer, 1998). By contrast, in stands of submerged vegetation due to plant nutrient uptake, nutrient concentration (N, P) of the water shows continuous decrease over the growing season (Scheffer, 1998; Szabó et al., 2022). Beyond the increasing temperature over the growing season, submerged vegetation itself can strongly modify the water temperature due to reduced turbulence and shading. Thus, over a longer growing period, the differences in temperature between the upper and lower water bodies are increasing. All in all, even in the absence of crowding of the vegetation, both abiotic (light, nutrient, and temperature) and biotic conditions are constantly changing, together with the inevitable change of physiological condition of the aging plants. Thus, in field conditions, it is not at all likely that the determined traits will remain stable over a longer growing periods. By contrast, in our experimental setup, under a shorter incubation period, we were able to keep the environmental factors nearly constant in order to gain relevant information regarding to the plant traits under changing environment in field conditions. Obviously, our experimental results may differ in several ways from the results of long-term studies under natural conditions. Natural pests and consumers may significantly change the competition between the two species (Koleszár et al., 2021). Furthermore, canopy formation of submerged macrophytes takes place along an increasing light gradient (from the shady bottom to the water surface); therefore, horizontal spread of the two species strongly depends on their apical elongation and branching degree along increasing light gradient (Szabó et al., 2019). Consequently, the differences in their phenotypic characteristics under various environmental conditions may also strongly determine their competitive outcome. Therefore, it is obvious, that our laboratory results do not

directly reflect the complexity of field conditions therefore, may not directly indicate the invasion success of *Cabomba*. However, the change in the observed traits in these controlled conditions is well consistent with the already-documented invasion of *Cabomba*. Thus, this study may help to understand further study of field traits of these species under more complex conditions.

Comparing all the results we may conclude, that H1 hypothesis was supported by the results that the trait differences for RGR and N:C molar ratio between species were more pronounced under decreasing light conditions and this likely contribute to the invasion success of *Cabomba* over *Myriophyllum*. Since *Myriophyllum* showed higher phenotypic plasticity for RGR, and *Cabomba* for SLA and LDMC, our second hypothesis has been proved to be also partly true. However, these characteristics make *Cabomba* a better survivor under turbid eutrophic conditions, contributing to the rapid spread of the species.

Based on these findings, shade tolerance seems to be a key factor in the invasion success of *Cabomba caroliniana*. In order to reveal more realistic image of the interplay between the two species, further long-term mesocosm experiments are needed to be performed cultivating them in cocultures.

AUTHOR CONTRIBUTIONS

Gergő Koleszár: Data curation (lead); formal analysis (lead); investigation (lead); methodology (equal); project administration (equal); software (equal); supervision (lead); validation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Balázs András Lukács:** Conceptualization (lead); data curation (supporting); formal analysis (lead); funding acquisition (lead); investigation (equal); methodology (supporting); project administration (equal); resources (lead); software (equal); validation (lead); visualization (equal); writing – review and editing (supporting). **Péter Tamás Nagy:** Data curation (supporting); methodology (supporting); software (lead). **Sándor Szabó:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); software (supporting); validation (supporting); writing – review and editing (supporting).

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DATA AVAILABILITY STATEMENT

The datasets related to this study are available at <https://datadryad.org/stash/share/2U0gbuswNQMg0AIlkj70mv0jhwzjZTOP7jbh8XHHp>.

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SUPPORTING INFORMATION

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Intraspecific trait variability is relevant in assessing differences in functional composition between native and alien aquatic plant communities

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Abstract The loss of plant functional diversity associated with biological invasion is a main subject of invasion biology, but still understudied in case of aquatic plants. We calculated functional richness, evenness, divergence and community-weighted mean trait values of aquatic plant communities for 20 plots, half invaded and half non-invaded by alien species, in a thermal effluent of West Hungary. Three traits [specific leaf area (SLA), leaf dry matter content (LDMC), leaf area (LA)] were considered to explain how alien species alter ecosystem function. We differentiated interspecific and intraspecific trait variation and investigate its effect on the community-level

functional diversity. We found that alien species invasion causes significant changes in the functional composition of aquatic plants whereby alien species become able to absorb light more efficiently, which will directly enhance their increased biomass production. Our study highlights the importance of local adaptation, showing that calculating functional diversity with global pooling of trait measurements causes significant over- or underestimation of functional diversity indices. Consequently, this can potentially result in erroneous conclusions regarding the impact of invasion.

Keywords Macrophytes · Community assembly · Functional diversity · Optimal pooling · Intraspecific trait variability

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Introduction

Most alien aquatic plants that have become invasive were planted deliberately in the new environment. Many of them can step quickly into the invasion phase and cause devastating effects (Getsinger et al., 2014; Brundu, 2015; Zhan et al., 2017). In temperate regions, biological invasion can be considered either advantageous or disadvantageous, their manner and degree are not consistent among ecosystems (Vilà & Hulme, 2017). Most recent approaches quantify the impact of alien species by documenting how it changes community species richness, phylogenetic

and functional diversity (Hejda & de Bello, 2013) or assessing the impact on ecosystem functioning (Vilà et al., 2011). It is widely accepted that alien plant species are associated with loss of species diversity in both terrestrial (Hejda & de Bello, 2013) and aquatic habitats (Vojtkó et al., 2017; Hussner et al., 2021). Since species contribute to the functional stability of communities, the replacement of native species inevitably modifies the stability and functioning of an ecosystem through the variability of traits (Vilà et al., 2011; Gallardo et al., 2016). Despite an increasing number of studies on functional diversity and biological invasion in the terrestrial realm, analogous studies in the freshwater environments with aquatic plants are few (Michelan et al., 2010). Indeed, comparing invaded and non-invaded sites using functional diversity indices and traits other than growth forms are still lacking.

Alien aquatic plants possess various traits which might contribute to their invasion success over native species like higher growth rate, better dispersal ability and higher propagule production (Hussner et al., 2021; Tasker et al., 2022 and references therein). Their superior resource acquisition (Lukács et al., 2017; Hussner et al., 2021), shade tolerance (Szabó et al., 2020; Koleszár et al., 2022) and phenotypic plasticity (Riis et al., 2012; Fleming & Dibble, 2015; Szabó et al., 2019) are also characteristics that make them better competitors than natives. Because of this competitive advantage, they often form monospecific stands, which alter ecosystem functions by changing community structure or role as habitat and food for animals (Dibble et al., 1996; Schultz & Dibble, 2012).

At the community level, changes in the functional composition can be characterized by mean and variation of traits measured by CWM (community-weighted mean) and functional diversity (FD) indices. CWM is expected to determine ecosystems process (such as biomass production) (Garnier et al., 2004), while FD has been related to resource use efficiency in plant communities.

According to the definition by Violle et al. (2012), a trait has to be measurable at the individual level; thus, traits vary among individuals both between (interspecific variation) and within (intraspecific variation) species. The extent of the latter variation differs between families or plant life forms but is known to be very high among aquatic plants (Willby et al.,

2000). It was traditionally assumed that interspecific trait variation is much larger than intraspecific variation (McGill et al., 2006; Shipley et al., 2016); therefore, the latter is often neglected, and each species represented by a single (mean) trait value (often downloaded from a database, Pavoine & Bonsall, 2011). However, this assumption is not generally met. Disregarding the within-species trait variation and applying a global mean trait value may distort the estimate of functional diversity and can lead to false conclusions. Thus, new approaches consider both between- and within-species variation (Carmona et al., 2016, 2019). Most aquatic plant researchers focus on community-level rather than on intraspecific trait variation (Veccia et al., 2020). Even if one decides to use intraspecific trait variation, it is not straightforward how it should be done. Carmona et al. (2019) proposed that first trait probability distributions have to be fitted, and subsequently, functional diversity indices should be calculated. But it is still questionable how data should be pooled before TPD fitting. Reliable estimates of distributions need a lot of data (cf. Appendix S1 in Botta-Dukát & Lukács, 2021). This fact suggests using all data irrespective of place of measurement to fit a single distribution. Botta-Dukát & Lukács (2021) pointed out that this approach may result in a biased estimate of TPD by neglecting within-species variation due to a changing environment. They proposed an algorithm for finding an optimal pooling with balance between increasing size (to improve the reliability of estimates) and homogeneity (to avoid bias due to neglecting the effect of the changing environment) of the trait dataset used in fitting a single TPD.

The aim of the present study was to assess the impact of biological invasion on the functional structure and diversity of native aquatic plant communities. To that, we hypothesized that (*H1*) alien species communities produce higher biomass, and the difference in the functional composition between native and alien communities reflects this. Specifically, we assumed (*H2*) that higher biomass production requires more efficient light capture and higher growth rates, and to achieve this, these species invest less in structural resistance.

Since aquatic plants display ubiquitous and remarkable environmental-induced phenotypic plasticity, we additionally analyse intraspecific trait variation. We followed the pooling methods given

by Botta-Dukát & Lukács (2021) and applied two types of trait pooling: optimal pooling of intraspecific trait variation (i.e. using species-specific trait probability distribution) and global pooling (using a global trait probability distribution). To that, we hypothesized (*H3*) that the optimal pooling provides different, and possibly more realistic results on functional diversity compared to global pooling.

Material and methods

Study area

This study was conducted in the Hévíz canal (West-Hungary), which is a natural, but canalized small river, with a constant water level. As the canal originates from a thermal lake (Lake Hévíz), it has an elevated water temperature of about 20 °C (yearly average). By this, even during the winter months, the temperature of the canal is rarely below 10 °C. In the 1980s, illegal aquarium plant cultivation was conducted in the upstream section of the canal; therefore, alien-invasive species could form dense mats, and the habitat structure is altered dramatically compared to the lower section where native vegetation remained uninvaded. In temperate regions, the ability to bypass the dormant period (i.e. overwintering) is an important environmental filter of alien species coming from the tropics. Freshwater habitats with elevated water temperatures provide excellent opportunities for cold-sensitive aquatic plant species to withstand the cold winter period and to get established even within the temperate region. For certain tropical and subtropical species, elevated water temperatures during winter might open “windows of opportunity”, it either enables successful overwintering in vegetative forms or ensures the survival of their seeds and buds throughout the winter season. Because of this, these plant species can form dense mats before the spring establishment of native aquatic plants, and these habitats are major sites of alien aquatic plant invasion in Europe (Šajna et al., 2007; Hussner et al., 2014a,b; Lukács et al., 2016), which makes them ideal study areas for testing functional diversity between alien and native vegetation.

Vegetation survey and environmental conditions

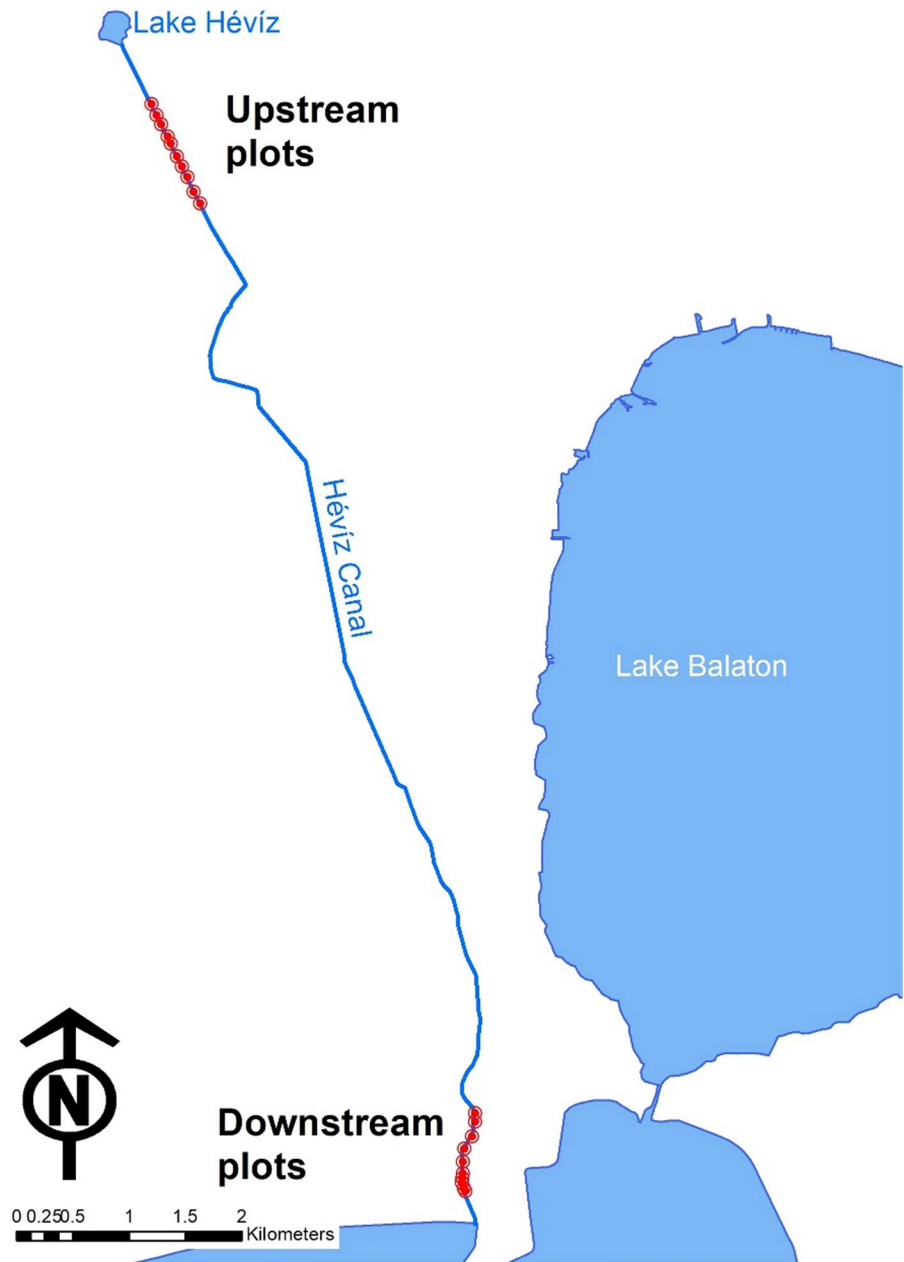
We designated 20 sampling plots (2 m×2 m each) along the canal. All plots were randomly assigned in the middle of the canal. As the distribution of native and alien aquatic plants is uneven along the canal, we assigned ten plots in the upper (thereafter upstream) and ten plots in the lower (downstream) section of the canal (Fig. 1).

The percentage cover of all vascular plants was recorded in each plot between September 9 and 11, 2017. Since there were pronounced overlaps between the species in the water column, we allowed the coverage to exceed 100%. After cover estimation, we assigned priority to collecting non-rooting species. Subsequently, we carefully cut the stems of rooting species just above the soil level and gathered the plant material within the plots for accurate biomass determination. We measured wet biomass per species and plot. In all plots, we measured temperature, pH and conductivity on site (Hach Lange HQ40D) and took water samples to measure total phosphorous and total nitrogen content. All measurement done in the environmental research laboratory at the Institute of Aquatic Ecology (ICP spectrophotometry).

Plant functional traits

We collected five young (photosynthetically active) but fully expanded leaves at the shoot tips from every plant species in all plots. Leaves without petioles were put into a zip-lock bag with a wet condition. Plant trait measurement and calculation followed the protocol of Pérez-Harguindeguy et al. (2013). Leaf fresh mass and leaf area were measured within 12 h of sampling using a scale (Adventurer Pro, Ohaus Inc.) and a benchtop leaf area meter (LI-3000C with LI-3050C, Li-COR Inc.). Leaf dry mass was measured after samples had dried at 80 °C for 48 h in a forced oven until a constant weight was achieved. Specific leaf area (SLA) was calculated as leaf area divided by its dry mass and expressed in mm² mg⁻¹. Leaf dry matter content (LDMC) was calculated as dry mass divided by the fresh mass of the same leaf and expressed in mg g⁻¹.

Fig. 1 Location of plots along the Hévíz Canal (West Hungary)



Functional diversity

We calculated *Community-weighted mean* trait values (CWM), which are the mean values of the traits across species weighted by their abundance (Violle et al., 2007), and were calculated for each plot ($n=20$). Besides CWM, we calculated different functional diversity indices to estimate trait differences between communities. We considered functional

richness, functional evenness and functional divergence (Mason et al., 2005; Villéger et al., 2008).

Functional richness

Functional richness measures how much of the niche space is occupied by the species present. Logically, functional richness is tightly connected to species richness.

Functional evenness

Functional evenness index measures whether the trait values are distributed regularly within the occupied trait space (a high index value usually reflects a very regular distribution; a low index value indicates aggregated distribution). Functional evenness is generally used to indicate under- or overutilization of resources and productivity, reliability and vulnerability to invasion (Mason et al., 2005).

Functional divergence

Functional divergence measures the degree to which the abundance of species in a community is distributed towards the extremities of occupied functional trait space.

Statistical analyses

Functional richness, evenness and divergence were calculated from species-level trait probability distributions (TPDs) with methods proposed by Carmona et al. (2016, 2019). Species level TPDs were fitted by two ways. In global pooling, all measurements were used for fitting a single TPD for each species, and these species-level TPDs were used for each plot. In optimal pooling, the approach proposed by Botta-Dukát & Lukács (2021) was applied: before fitting TPDs, plots were classified into groups by fitting a Gaussian finite mixture model, then separate TPDs were fitted for each group. Functional diversity analyses made under R environment with the use of *ks* (Duong et al., 2022), *mclust* (Scrucca et al., 2016) and *TPD* (Carmona, 2019) packages. CWMs and functional diversity indices of invaded and uninvaded plots were compared by Wilcoxon tests. Figures and Wilcoxon test were made in Origin Pro 2023 software (OriginLab Corporation, Northampton, MA, USA).

Results

We found significantly higher temperature, higher total phosphorous content, higher conductivity and lower pH in the water at the upstream section (Wilcoxon test, Table 1). We recorded eight alien and five native species in the plots (Table 2). Alien species were exclusively found in the upstream plots,

Table 1 Values of environmental variables in Hévíz canal, and their differences between upstream and downstream sections

	Upstream section (mean ± S.E.)	Downstream section (mean ± S.E.)	diff
Temperature (°C)	26.45 ± 0.283	20.03 ± 0.249	*
Conductivity (µS/cm)	720.6 ± 4.01	680.6 ± 2.490	*
pH	7.279 ± 0.024	7.705 ± 0.052	*
TN (mg/l)	0.367 ± 0.04501	0.3172 ± 0.0446	
TP (µg/l)	87.272 ± 8.292	50.909 ± 7.241	*

* $P < 0.05$ (Wilcoxon test)

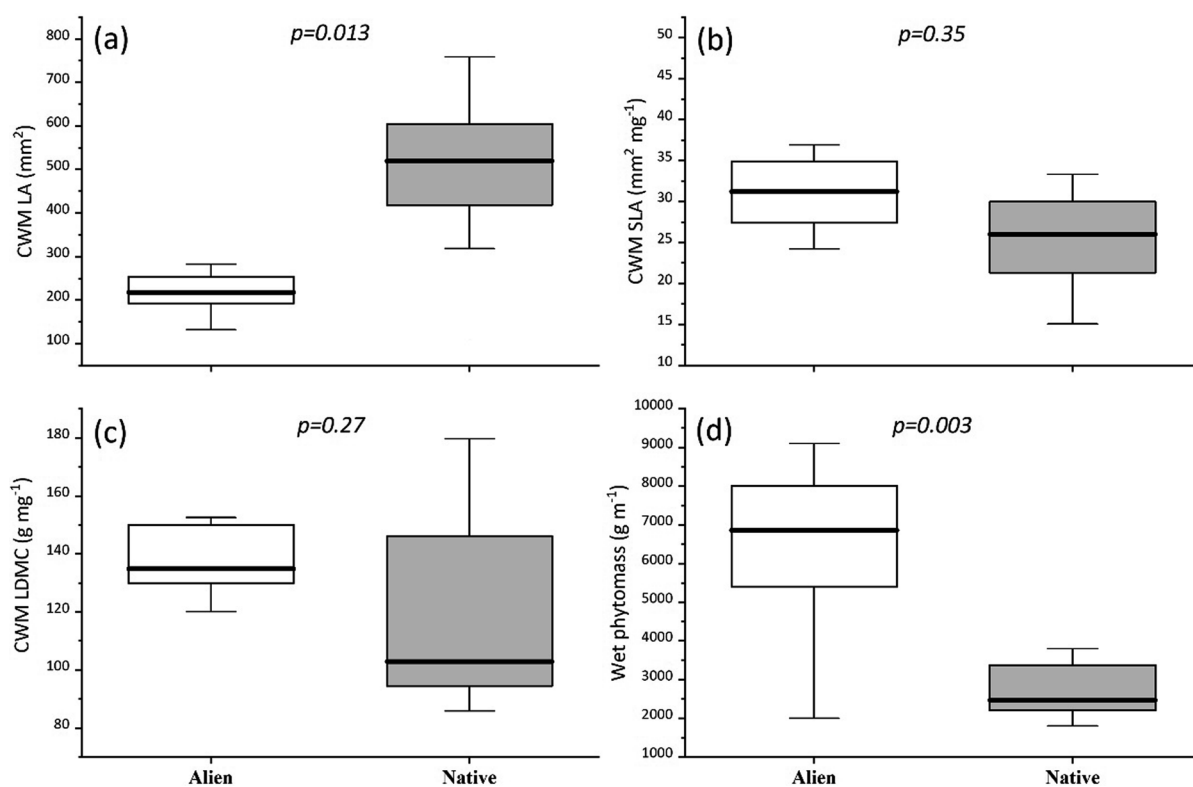
while native species exclusively found in the downstream plots. We measured significantly lower wet biomass in communities dominated by native species (Fig. 2d). Community-weighted mean values of LA were significantly higher, but mean values of SLA were significantly lower in communities dominated by native species (downstream plots) (Fig. 2a).

Global pooling always significantly overestimated the functional richness, irrespective of trait and community type (Table 3, Figs. 3, 4, 5). In alien communities, the functional divergence of SLA and LDMC was significantly underestimated by global pooling, but the effect of pooling was not significant in native communities and in the case of LA. Estimates of functional evenness by two ways of pooling did not differ significantly.

Using global pooling, the functional richness and evenness of LA, and the functional evenness of LDMC and SLA were significantly higher in alien communities, while functional richness of SLA and functional divergence of the LA were significantly higher in native communities (Figs. 3, 4, 5). Using optimal pooling, only four of these six significant differences—the higher functional richness and evenness of LA, the higher functional evenness of LDMC and the lower functional divergence of LA in invaded communities—could be confirmed. In the other two cases, the difference between invaded and native communities was not significant when optimal pooling was applied instead of global one, and one new significant difference—higher functional divergence of SLA in the native communities—emerged.

Table 2 List of aquatic plant species and their major growth-form found in the plots

	Species	Growth-form
Alien	<i>Cabomba caroliniana</i> A.Gray	Submerged/rooted
Alien	<i>Hygrophila difformis</i> (L.f) Blume	Submerged/rooted
Alien	<i>Nymphaea lotus</i> L	Floating leaved/rooted
Alien	<i>Nymphaea rubra</i> Roxb.ex Andrews	Floating leaved/rooted
Alien	<i>Rotala rotundifolia</i> (Buch.-Ham. ex Roxb.) Koehne	Partly emerged/rooted
Alien	<i>Vallisneria americana</i> Michx	Submerged/rooted
Alien	<i>Vallisneria spiralis</i> L	Submerged/rooted
Native	<i>Ceratophyllum demersum</i> L	Submerged/non-rooted
Native	<i>Hydrocharis morsus-ranae</i> L	Floating leaved/non-rooted
Native	<i>Lemna minor</i> L	Floating leaved/non-rooted
Native	<i>Nuphar lutea</i> (L.) Sm	Floating leaved/rooted
Native	<i>Spirodela polyrhiza</i> (L.) Schleid	Floating leaved/non-rooted

**Fig. 2** Boxplots showing variation of community-weighted mean values of **a** leaf area (LA), **b** specific leaf area (SLA), **c** leaf dry matter content (LDMC) and **d** variation of wet phytomass of native and alien communities. $P < 0.05$ indicates

significant differences between native and alien communities (Wilcoxon test). Boxes show interquartile ranges, middle lines are medians and whiskers are outliers

Table 3 Wilcoxon-test results for comparing the effect of global and optimal pooling using invasive and native communities for estimating trait probability distributions (TPD)

Trait	FD measure	Invasive communities	Native communities
SLA	Richness	$W=77$ P value = 0.0444	$W=88$ P value = 0.0041
	Evenness	$W=36$ P value = 0.315	$W=39$ P value = 0.4359
	Divergence	$W=16$ P value = 0.0089	$W=36$ P value = 0.315
LDMC	Richness	$W=78$ P value = 0.0372	$W=82$ P value = 0.01561
	Evenness	$W=25$ P value = 0.0630	$W=39$ P value = 0.4359
	Divergence	$W=18$ P value = 0.0147	$W=49$ P value = 0.9705
LA	Richness	$W=90$ P value = 0.0028	$W=100$ P value = 0.0002
	Evenness	$W=55$ P value = 0.7394	$W=55$ P value = 0.7394
	Divergence	$W=70$ P value = 0.1431	$W=40$ P value = 0.4813

Significant differences ($p < 0.05$) between values calculated by global and optimal pooling are in bold

Discussion

Our study aimed to evaluate the effect of biological invasion on the functional structure and diversity of aquatic plant communities within the river. Additionally, we sought to examine the variations in functional diversity indices by applying trait probability distributions fitted using both the global pooling and optimal pooling methods. We showed that invasion significantly changed the functional composition of aquatic plant communities and altered their ecosystem functioning expressed by biomass production in a thermal effluent. Our study also raised the importance of intraspecific trait variability in the community-level analyses of functional diversity among aquatic plants.

Functional composition and productivity

Invasive alien species have been usually linked to higher growth rate, and higher biomass production, which allows them to outcompete native species

(Dawson et al., 2010). However, this feature depends on the studied species and habitats. In accordance with our first hypothesis, we found that alien aquatic plant communities produced significantly higher biomass compared to native ones. We also assumed that more efficient light capture is required for such an increased biomass production and suggested a shift in the community composition towards the dominance of larger-leaved species. In contrast, our findings revealed lower community-weighted mean LA values within alien communities. This indicates that these communities are comprised of species that possess having either smaller or more dissected leaves (i.e. higher surface–volume ratio), or even a combination of both, in comparison to natives. We assumed that alien species may overshadow themselves due to the denser vegetation caused by larger phytomass production. In such dense vegetation, the smaller-dissected leaved species may have an advantage, as these leaves are easier to position for more optimal light capture (Ritchie & Olff, 1999; Poorter & Roozendal, 2008). Therefore, smaller-dissected leaved species can achieve an advantage in the competition for resources such as light. The higher functional richness of LA in alien communities indicates a wider range of leaf sizes within the community, which may contribute to ecosystem resilience and stability by ensuring more efficient light capture (Naem, 1998). On the other hand, the lower functional divergence of LA in alien communities corroborates the aforementioned findings. This suggests that alien species may exhibit a higher degree of functional redundancy in leaf size, i.e. the most abundant species have consistently small leaves.

SLA is considered to be an important component of the leaf economic spectrum; it characterizes the efficiency with which leaves allocate nutrients and dry mass, influencing various ecosystem processes, particularly primary production (Wright et al., 2004). We did not find differences in the community-weighted mean values of SLA between alien and native communities and this result was also confirmed by similar functional diversity and evenness values. This is in line with Gustaffson & Norkko (2019) who found that SLA has only indirect influence on primary production of aquatic plants. Since SLA is an important component of relative growth rate (RGR) (Westoby, 1998), our results suggests that alien species do not differ from native species in their growth rate strategy.

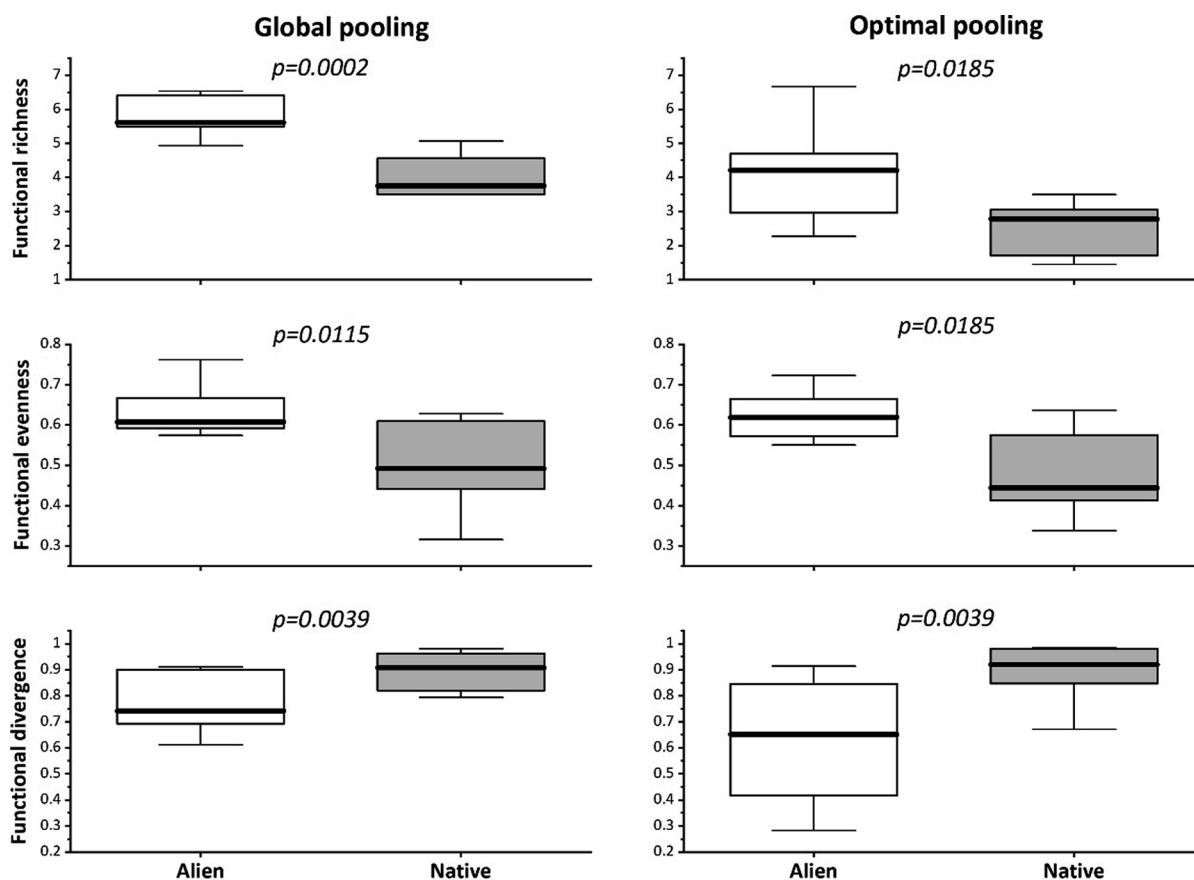


Fig. 3 Boxplots of functional richness, evenness and divergence in leaf area (LA) in native and alien communities calculated by global and optimal pooling of trait data. $P < 0.05$

Hussner et al. (2021) gave a comprehensive picture of the growth rate strategy of invasive aquatic plants and its dependence on habitats, biotic and abiotic factors. Our results clearly indicate that trait differences of native and alien species cannot be detected at the community level, regardless of its impact at the species-level (Lukács et al., 2017).

In accordance with these results, lower functional divergence values of alien communities reveal an overall functional homogenisation of invaded communities (Castro-Díez et al., 2016), while the significantly lower values of LA and SLA indicate reduced competition for light and nutrients compared to native communities (Ordonez et al., 2010). Contrary to our hypotheses, alien and native communities did not differ in the community-weighted mean values of LDMC, which means that alien and native communities in general possess the same

indicates significant differences between native and alien communities (Wilcoxon test). Boxes show interquartile ranges, middle lines are medians and whiskers are outliers

ability to resist physical damages. Leaf dry matter content is assumed to be correlated with palatability via plant nutrient content both in terrestrial (Pake-man, 2014) and aquatic plants (Elger & Willby, 2003; Elger & Lemoine, 2005; Zhang et al., 2019). Considering this, we conclude that alien communities do not have better resistance to herbivory, thus they might have the same top-down control possibilities than native aquatic plants (O'Connor, 2009). The same functional diversity and functional divergence values of LDMC in native and alien communities confirms this assumption. LDMC has been proposed to be the best single variable for predicting species resource use efficiency (Wilson et al., 1999). Yet our results showing no difference for this trait might indicate that alien and native communities do not differ in their resource use efficiency, which led us to conclude that alien species do not

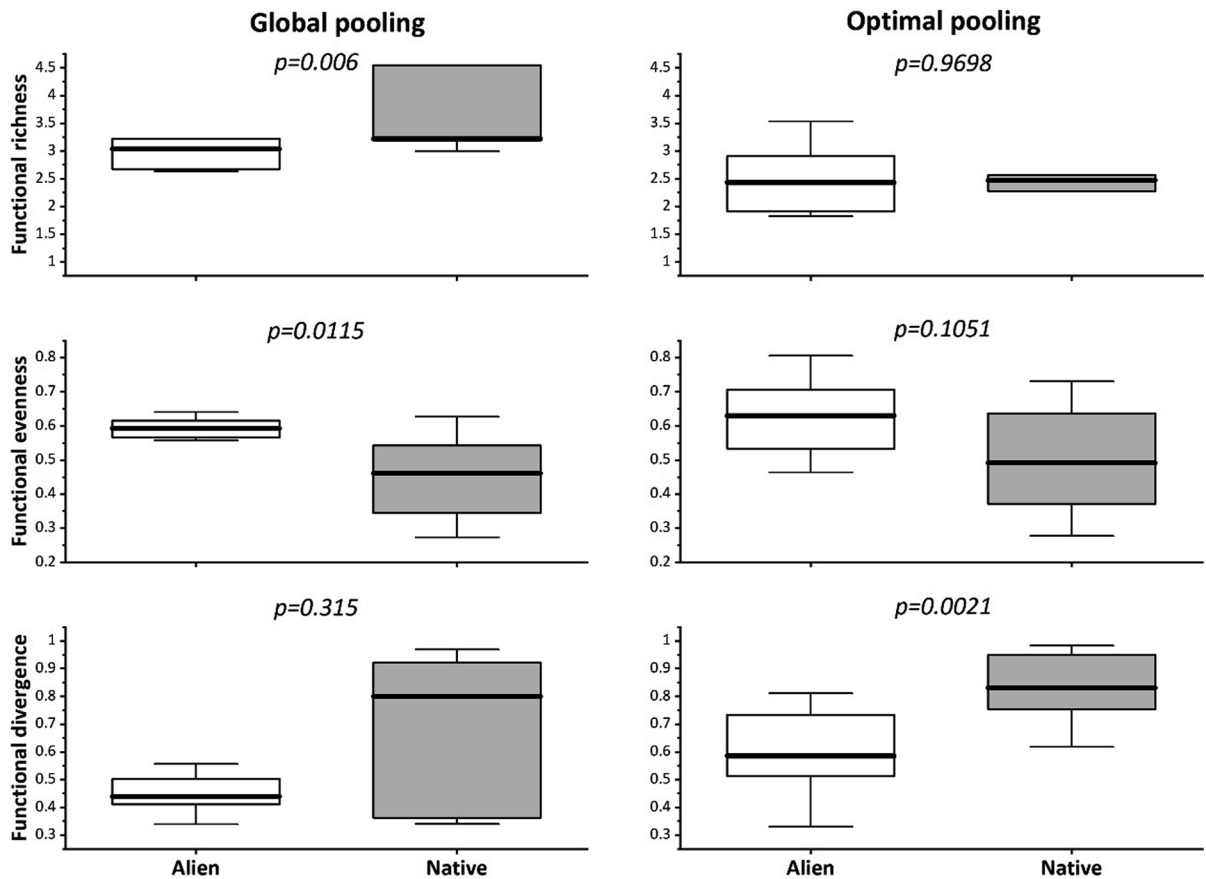


Fig. 4 Boxplots of functional richness, evenness and divergence of specific leaf area (SLA) in native and alien communities calculated by global and optimal pooling of trait data.

$P < 0.05$ indicates significant differences between native and alien communities (Wilcoxon test). Boxes show interquartile ranges, middle lines are medians and whiskers are outliers

alter this part of ecosystem services. However, this conclusion must be qualified with an important caveat, because this correlation has not yet been validated for aquatic plants.

In our study, models (global pooling and optimal pooling) revealed that functional evenness of aquatic plant communities is significantly higher in alien dominated communities for all traits investigated. In other words, trait values tend to be more evenly distributed in the alien communities in terms of their abundance and dispersion in the functional trait space. This could be an indication that niche differentiation (via limiting similarity) is driving (or more enhanced) the invaded community assembly (Kraft et al., 2008; Aschehoug & Callaway, 2015). Given the significant higher biomass production of

alien communities, we suggest that alien communities have higher evenness in order to maximize biomass production via niche complementarity (Tilman et al., 2014). Theory also predicts that high evenness maximizes the so-called “portfolio effect”, whereby communities can achieve a higher stability to respond to environmental fluctuations (Oliver et al., 2015). Accordingly, higher functional evenness of LA among alien macrophytes may ensure a community to achieve a temporarily stable (hence more efficient) light capture required for increased primary productivity (Lukács et al., 2019). In contrast, significantly higher evenness of LDMC in alien communities might ensure a better resilience of the community through enhanced physical resistance (e.g. to herbivory, wind, currents), longer leaf

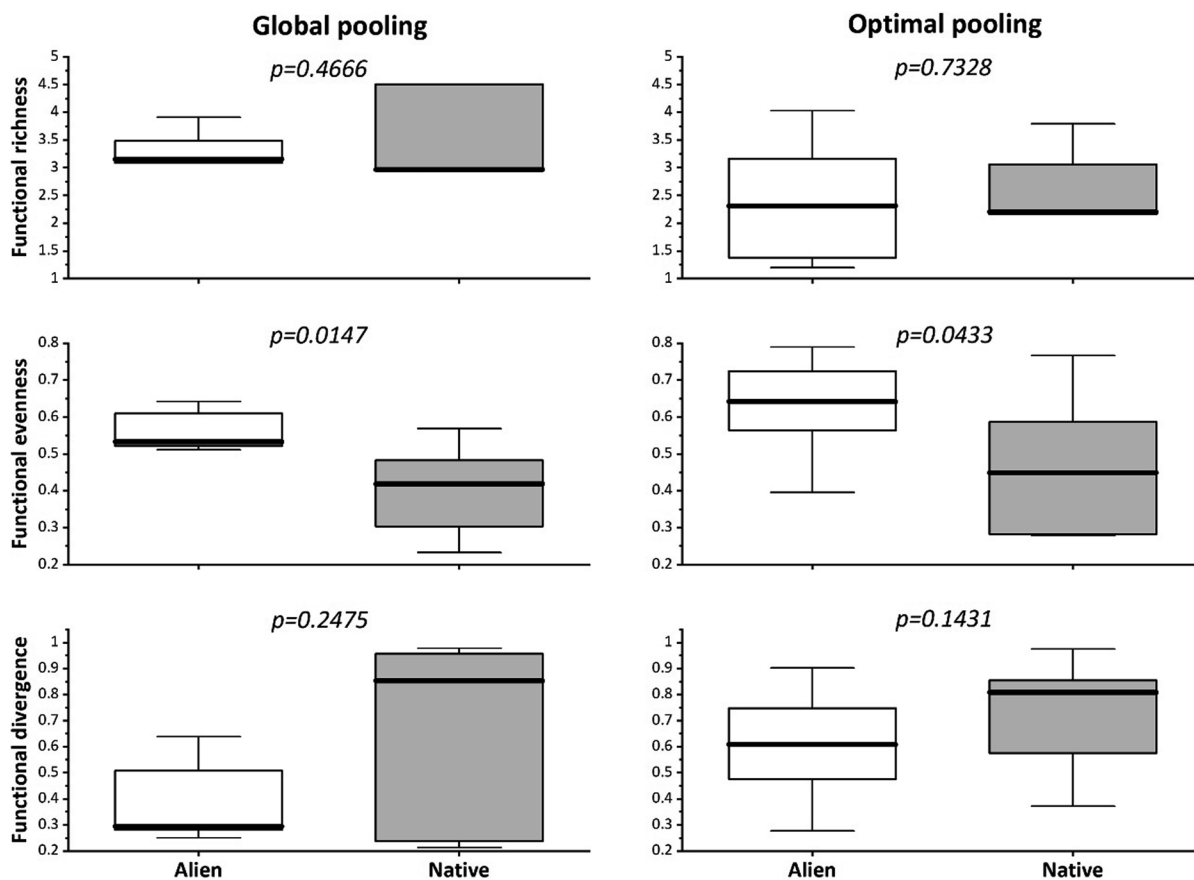


Fig. 5 Boxplots of functional richness, evenness and divergence of leaf dry matter content (LDMC) in native and alien communities calculated by global and optimal pooling of trait

data. $P < 0.05$ indicates significant differences between native and alien communities (Wilcoxon test). Boxes show interquartile ranges, middle lines are medians and whiskers are outliers

lifespan and better resource use efficiency (Pérez-Harguindeguy et al., 2013).

Effect of pooling species

To our knowledge, nobody has so far compared functional diversities for plants in general calculated from TPDs fitted with these two different pooling approaches. The difference between the two pooling approaches was higher in invaded than in native communities. This suggests that trait distributions of invasive species vary more strongly among sites. This might be due to their higher environmentally structured genetic diversity, higher phenotypic plasticity or both. Effect of genetic diversity and phenotypic plasticity could be

separated only by common garden experiments, but both could enhance the invasiveness of alien species (Fleming & Dibble, 2015; Hussner et al., 2021 and literature therein). In general, functional richness is overestimated, while functional divergence is underestimated by global pooling. The former can be explained by pooling trait values adapted to different environments. On the other hand, underestimation of functional divergence is an unexpected pattern, probably related to site-specific separation of species in the niche space. The functional evenness proved to be the less sensitive to the applied pooling approach. The over- and underestimations influenced the outcomes of comparison of native and invasive communities: two of the six significant differences disappeared, and a new significant difference emerged when pooling changed from global

to optimal. These results question the reliability of results based only on global pooling.

Conclusion

The present study demonstrates for the first time that the invasion of alien aquatic plants causes a significant impact not only on species diversity but on functional diversity of the recipient community. With the use of functional diversity indices, we point to the functional mechanism leading to the higher biomass production of alien communities. Changes in the functional composition of communities usually have a significant impact on the quality of ecosystem functions e.g. reduce ecosystem resilience leading to transformations, that affect fish population that can also have a negative effect on food security; it alter flow rates, which reduce water quality; hamper their use in tourism. Measures to mitigate their effects often lead to increased management costs and efforts (Catford, 2017; Shackleton et al. 2018). We showed that alien aquatic plants produce significantly higher biomass, and this increase in production is obtained through a set of adaptations such as smaller leaves, dissected leaves, leaf positioning which allow these species to form large dense stands. Such dense mats of alien species can strongly decelerate water flow, causing negative effect on fishes and macroinvertebrates (Tasker et al., 2022).

Our study highlights that the use of intraspecific trait variation can provide much deeper insights not just in studies on plant invasion but in any kind of functional diversity analyses. Considering the rich within-species variation of aquatic plants influenced by a changing environment yields remarkably diverse functional outcomes, fostering a deeper understanding and more accurate conclusions.

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Data availability All data is available upon request from the authors.

Declarations

Conflict of interest There is no conflict of interest.

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