



# The non-native charophyte *Nitellopsis obtusa* (starry stonewort) influences shifts in macrophyte diversity and community structure in lakes across a geologically heterogeneous landscape

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**Abstract** *Nitellopsis obtusa* is a non-native charophyte introduced to the Laurentian Great Lakes in the 1970s. Over the last decade, there have been increased reports of *N. obtusa* invasion of inland lakes in the Great Lakes basin. Typically, the star-shaped bulbil associated with *N. obtusa* can aid in identification; however, bulbils can only be found in established communities. To evaluate the impacts of *N. obtusa* on macrophyte communities in invaded ecosystems, we sampled 60 lakes across a large geologically diverse area of south-central Ontario. Interestingly, all populations of *N. obtusa* detected had bulbils present, thus we conclude that *N. obtusa* has likely been present in inland lakes within the Great Lakes basin well beyond the last decade. High *N. obtusa* abundance was associated with significantly lower macrophyte diversity ( $p$  value < 0.05). Redundancy analysis revealed that increased developed and agricultural land-use, in addition to the cations: sodium,

potassium, magnesium, and calcium, positively covaried with macrophyte communities with detectable *N. obtusa* presence. A generalized linear latent variable model (gllvm) demonstrated that *N. obtusa* had significant ( $p$  value < 0.05) positive co-occurrences with several native Characeae species. The gllvm model also revealed that *N. obtusa* had significant ( $p$  value < 0.05) negative co-occurrences with most other macrophytes, including the non-native *Myriophyllum spicatum*. Although *N. obtusa* was only found in a third of our study lakes, our results infer that increased expansion and establishment of *N. obtusa* throughout the region may result in significant changes to macrophyte communities, posing a threat to native fish habitat.

**Keywords** Characeae · Invasive species · Macrophyte community · Gllvm · Species co-occurrence

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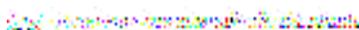
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## Introduction

*Nitellopsis obtusa* (Desv.) J. Groves, 1919, commonly referred to as starry stonewort, is a non-native species in North America belonging to the Characeae family. The native distribution of *N. obtusa* extends from Britain through most of Europe and Asia to Japan. Over 80% of Characeae taxa, including *N. obtusa*, are now considered endangered in their native ranges due to climate change and persistent anthropogenic



stressors (Blaženčić et al. 2006). Throughout its native range, *N. obtusa* is classified as threatened or endangered (Hamann and Garniel 2002; Blaženčić et al. 2006; Caisová and Gabka 2009; Johansson et al. 2010; Kabus and Mauersberger 2011; Auderset Joye and Schwarzer 2012; Korsch et al. 2013; Kato et al. 2014). Although no systematic studies have been done to compare native and non-native habitats, Escobar et al. (2016) noted that the environmental characteristics of North American and Eurasian distribution ranges were similar. Thus, the invasive tendency of *N. obtusa* within North America may reflect release from native habitat constraints such as competition and predation.

How *N. obtusa* came to North America is not known; however, it has been conjectured that accidental introduction occurred via the ornamental gardening trade and trans-oceanic shipping (Kay and Hoyle 2001; Padilla and Williams 2004). The first record of *N. obtusa* in North America is a specimen collected from the St. Lawrence River cataloged at the New York Botanical Garden, New York, USA, in 1974 (Karol and Sleith 2017). Over the intervening period, *N. obtusa* did not emerge as an invasive species threat in inland waters of the Great Lakes basin until the mid-2000s (Larkin et al. 2018). In Ontario, *N. obtusa* has been documented in Lake Simcoe as early as 2008 (Ginn et al. 2021), Presqu'île Bay of Lake Ontario since 2015 (Midwood et al. 2016), and Lake Scugog as early as 2015 (Harrow-Lyle and Kirkwood 2020a, b). Recently, we confirmed *N. obtusa* establishment in 19 lakes across south-central Ontario (Harrow-Lyle and Kirkwood 2021a).

The common name of starry stonewort originates from the conspicuous white star-shaped bulbils. These bulbils arise from the rhizoid nodes and monofilament, and serve as hibernation cells and asexual reproductive structures (Bharathan 1987). Despite the presence of bulbils being an important diacritical feature for distinguishing between Characeae, identification solely on the presence of bulbils may prove difficult, as bulbils are only found within established beds of *N. obtusa* (Bonis and Grillas 2002). When bulbils are absent, *N. obtusa* can be identified by its long and slender thalli that are typically encrusted in a thick marl, composed of calcium carbonate and phosphorus (Blindow 1992). Branchlets are arranged in 5–8 per whorl, and grow upward of 9 cm in length (Karol and Sleith 2017). Furthermore, in spite of other

Characeae such as *Chara* spp., giving off a characteristic sulfurous odor, *N. obtusa* does not exude a prominent smell (Pullman and Crawford 2010; Hackett et al. 2014). Generally, *N. obtusa* resembles *Nitella* spp. and *Chara* spp. due to the whorled branches that arise from stem nodes. The high degrees of similarity between *N. obtusa* and native Characeae make detection difficult, leading to repeated misidentification and underreporting of this invasive taxon.

The threat that *N. obtusa* poses toward North American freshwater ecosystems is thought to be similar to other non-native macrophytes (Pullman and Crawford 2010; Hackett et al. 2014; Brainard and Schulz 2017). Pullman and Crawford (2010) concluded after several years of lake monitoring that *N. obtusa* was the most aggressive macrophyte ever observed within Michigan, United States, yet the invasion biology of *N. obtusa* in North America is largely unknown. Few studies have documented community- to ecosystem-level impacts of *N. obtusa*, from early colonization to full establishment. However, some studies have observed displacement of other macroalgae and vascular aquatic plants upon *N. obtusa* establishment (Pullman and Crawford 2010; Brainard and Schulz 2017; Ginn et al. 2021; Harrow-Lyle and Kirkwood 2020b, 2021c).

To improve our understanding of *N. obtusa* impacts on macrophyte communities, we surveyed 60 lakes across Ontario, Canada, to (1) Document the occurrence and abundance of *N. obtusa* and (2) Evaluate macrophyte community composition and diversity as a function of environmental conditions and *N. obtusa* biomass. In previous work (Harrow-Lyle and Kirkwood 2021a) we were able to elucidate the key abiotic habitat conditions constraining and promoting *N. obtusa* presence in invaded lakes. Here, we not only wanted to characterize the macrophyte community composition in invaded lakes, but we also wanted to tease apart the relative influence of *N. obtusa* on community structure when abiotic variables could be accounted for using a generalized linear latent variable modeling (gllvm) approach. The study area stretches across a notable geological mosaic, where the limestone-dominated bedrock of the St. Lawrence lowlands shifts to the granite-dominated bedrock of the Precambrian shield. By sampling over such a geologically diverse landscape, we were able to capture a wide range of habitat conditions reflecting broad environmental gradients.

## Materials and methods

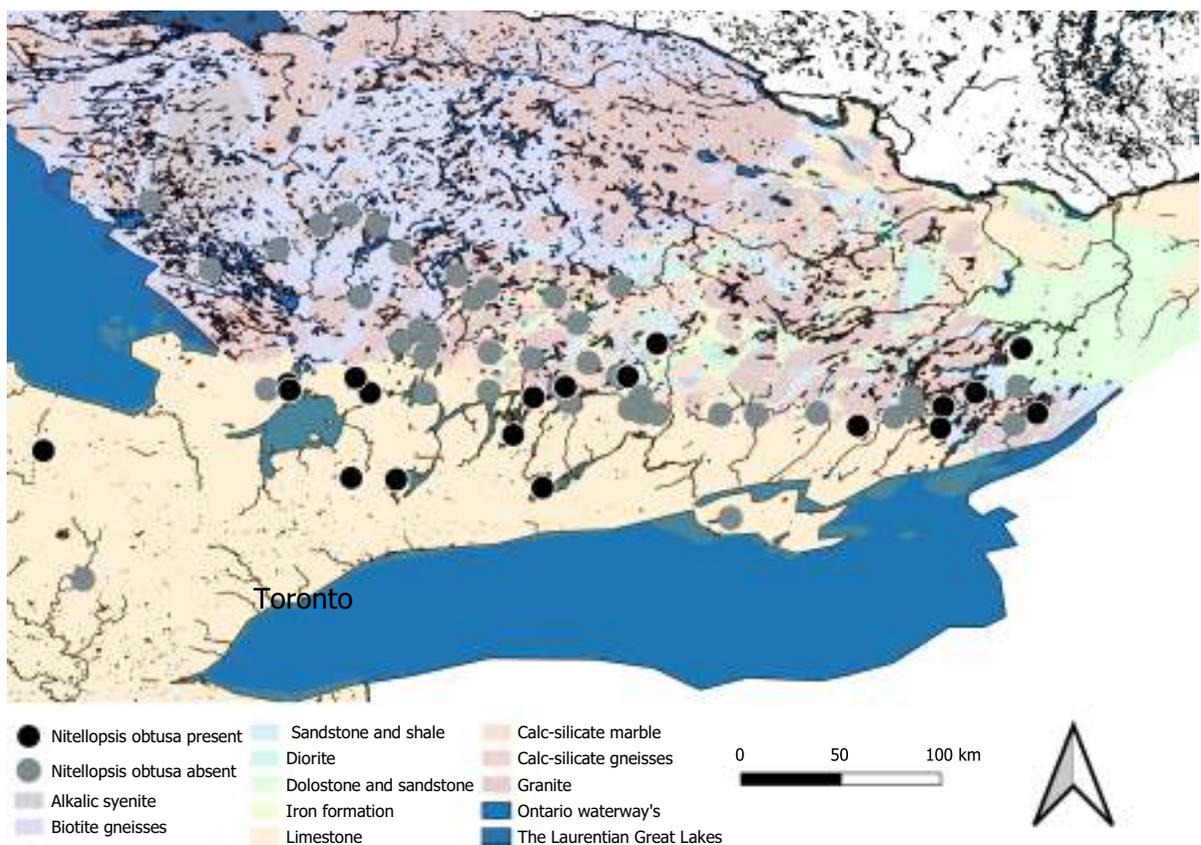
### Study design

A survey of 60 lakes spanning a large geographic region (~65,000-km<sup>2</sup>) in Ontario, Canada, was conducted within a narrow time frame (August 1, 2019, to August 22, 2019) to minimize seasonal effects. Candidate lakes that were investigated are outlined in Harrow-Lyle and Kirkwood (2021a) (Fig. 1). Briefly, lake selection was based on capturing a broad water hardness gradient using historical calcium data collected from a variety of sources (The Land Between Charity, Kawartha Conservation, and the Ontario Ministry of Environment, Conservation, and Parks), while also minimizing surface connectivity through Geographic Information System (GIS) analysis (QGIS Development Team 2016). A transect which

ran perpendicular to the shoreline was identified using bathymetric maps (e.g., Anglers Atlas <https://www.anglersatlas.com>) and was sampled within each of the candidate lakes. Four sampling sites were distributed along each transect to capture a range of depths (0.5 to 15 m) in each lake. At each location along the depth transect, water and macrophyte communities were sampled.

### Sample collection and field measurements

Depth at each site was verified using a Hawkeye handheld depth finder (DT1H, Hawkeye Electronics, Stuart, Florida, USA). Water clarity was measured using a standard 20-cm diameter Secchi disk. Conductivity, dissolved oxygen, temperature, and pH field measurements were recorded at each site using a YSI 6-series multiparameter probe (YSI Inc., Yellow



**Fig. 1** Map of candidate lakes with underlying geology. Candidate lakes are shown as circles, where black circles denote lakes with *N. obtusa*, and gray circles are lakes where *N.*

*obtusa* was not documented. Underlying geology shape files were obtained from the Ontario Geological Survey (2011), which represent a 1: 250 000 scale in bedrock geology

springs, Ohio, USA). For consistency, water samples were collected at 0.5 m using a Van Dorn Sampler. Samples were decanted into four 50-mL conical tubes that were previously acid washed to remove trace nutrients and contaminants. Following collection, samples were immediately placed on ice until they were transferred to a cold room facility at Ontario Tech University at the end of each sampling day. Macrophyte collection followed the lake-rake method of Ginn (2011). At each site, three rake throws that extended to the lakebed were performed by the same person in following a consistent protocol. All material captured was placed in extra-large Ziplock bags and placed in a cooler on ice.

### Sample processing

Samples were processed within 24 h of collection. Total phosphorus (TP) concentrations were determined following the modified ascorbic acid method of Murphy and Riley (1962) that was developed by the Ontario Ministry of Environment (1983). Total organic carbon (TOC) and total nitrogen (TN) were measured using a combustion catalytic oxidation method at York University, Toronto, Canada (TOC5000 series TOC analyzer, Shimadzu, Kyoto, Japan). Samples were sent to an accredited laboratory (SGS Canada, Lakefield, Ontario) for cation suite analysis that included: calcium, magnesium, sodium, potassium, iron, and manganese. Macrophytes were washed with reverse osmosis water, sorted, and identified using a QZE stereomicroscope (Walter Products, Windsor, Ontario). Samples were dried in a convection oven at 80 °C for 24 h following Carr et al. (2003). Biomass of each macrophyte species was determined by dry weight of collected material (g).

### Statistical analysis

Statistical analyses were done using the open-sourced platform R version 4.0.3 (R Core Team 2019). Trophic status was inferred by calculating a trophic state index using TP as previously described (<https://www.nalms.org/secchidipin/monitoring-methods/trophic-state-equations/>). Macrophyte taxa abundance and environmental parameters were averaged for each lake ( $n=4$ ). Macrophyte Simpson's diversity (1-D) was calculated for each lake using the R package vegan (Oksanen et al. 2013). *Nitellopsis obtusa*

biomass was removed from the macrophyte community matrix prior to Simpson diversity calculations. Diversity for each candidate lake with confirmed *N. obtusa* populations was split into two categorical classifications; low *N. obtusa* presence (< 50% of relative abundance) and high *N. obtusa* presence (> 50% of relative abundance). A Mann–Whitney–Wilcoxon test was used to determine whether Simpson's diversity significantly differed between the categorical classifications.

Redundancy analysis (RDA) was used to visualize the variation in macrophyte community structure using log-transformed macrophyte dry-weights. RDA was chosen based on the results of a detrended correspondence analysis, which found the longest gradient to be < 3. Biplot scores are representative of the weighted sums of species, as this measure is robust to noise within environmental variables. RDA analysis was performed using a Bray–Curtis dissimilarity matrix. The resulting RDA was prepared using the R packages stringr (Wickham 2019), dplyr (Wickham and Francois 2016), tidyr (Wickham and Henry 2019), vegan (Oksanen et al. 2013), and plotted in ggrid (Beck 2016). A permutational analysis of variance (PERMANOVA) was used to test if there was a statistical difference between communities with (low and high) and without *N. obtusa* present (Oksanen et al. 2013).

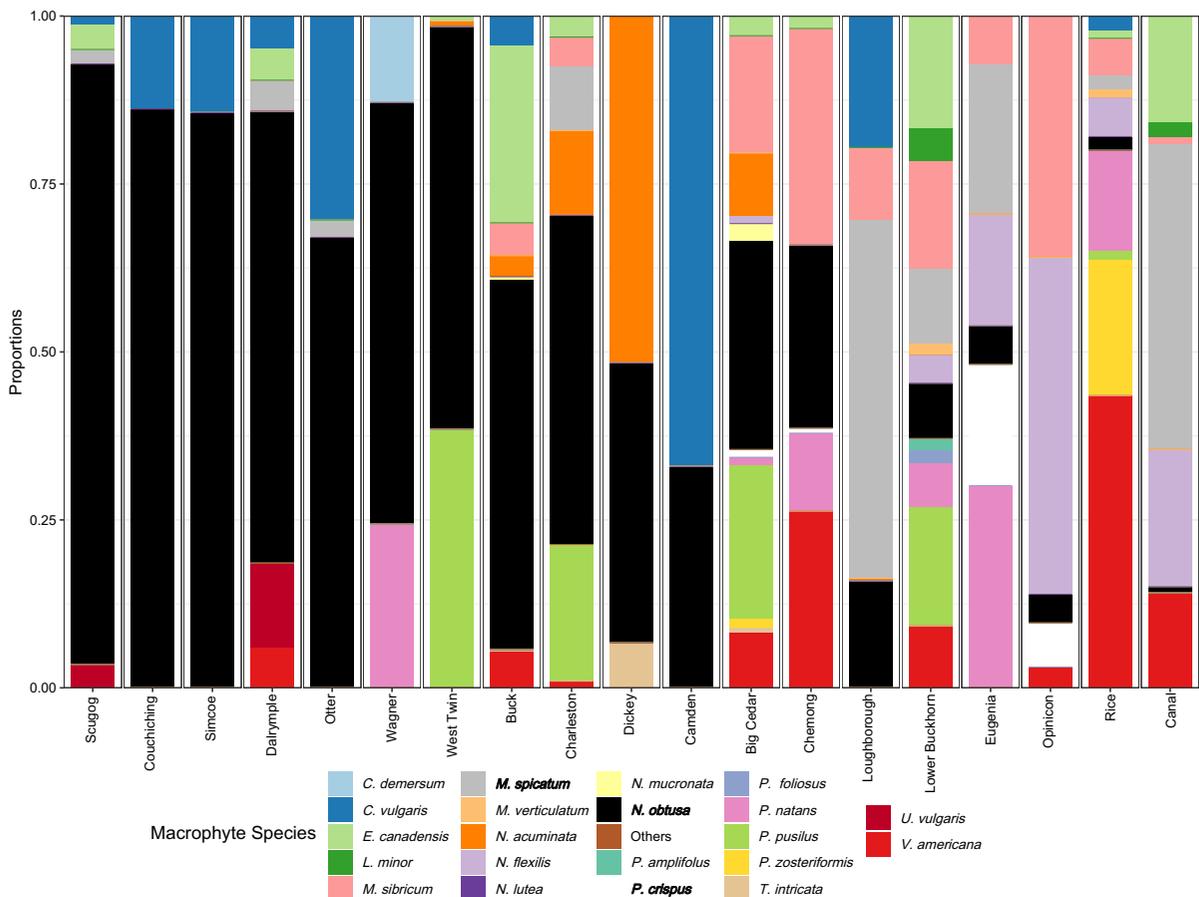
Although an assessment of macrophyte species diversity revealed general community patterns, diversity alone does not address individual relationships. Understanding interactions between taxa within a community, especially in response to an invading taxon, are often difficult and complex to quantify. A relatively new multivariate approach that was developed to address this issue is generalized linear latent variable modeling (gllvm). An important advantage of this modeling technique is its ability to handle many species at once compared to other methods, and because the covariance model scales linearly (Warton et al. 2015).

The resulting gllvm model was constructed following Niku et al. (2019). Poisson, Tweedie, zero-inflated Poisson, and negative binomial distributions were fit to macrophyte abundance data (dry weight g). Information criterion, Dunn–Smyth residual plots, and normal quantile–quantile plots with 95% confidence intervals were used to assess the fit for macrophyte community distributions (Niku et al. 2019).

A negative binomial distribution was identified to be the best fit for the data and was used in the following analysis. Environmental parameters were added into the model, to account for the effects on species interactions. A model that used five of the water quality parameters (calcium, potassium, pH, manganese, and depth) was chosen based on residual analysis indicating the most suitable mean–variance relationship for the responses. Covariance stored in the residual matrix that is not explained by the water quality parameters were calculated and adjusted to account for the variation explained by the negative binomial distribution, as outlined by Niku et al. (2019). Correlation patterns for species-specific relationships were plotted in R using the corrplot, RColorBrewer, and gclus packages (Hurley 2005; Neuwirth 2014; Wei et al. 2017).

### Results

General lake characteristics, dominant submerged vegetation, and trophic status are displayed in supplementary table 1 (Table S1). *Nitellopsis obtusa* was found in 19 of the 60 study lakes (Fig. 2). The community proportion that *N. obtusa* comprised in each of the invaded lakes varied from < 1 to 90% (Fig. 2). Lakes Scugog (90%), Simcoe (86%), and Couchiching (86%) had the highest proportion of *N. obtusa* biomass (Fig. 2). In contrast, lakes Canal (0.89%), Rice (2.1%), and Opinicon (4.3%) had the lowest proportions of *N. obtusa* biomass in this study (Fig. 2). The remaining thirteen study lakes had *N. obtusa* at variable proportions (5.7 to 67%) of the total macrophyte community (Fig. 2). In the low *N. obtusa* presence classification, macrophyte diversity was



**Fig. 2** Macrophyte community taxa plots for the 19 candidate lakes where *N. obtusa* was identified across south-central Ontario. Communities were averaged between the four sites per candidate lake. Relative proportions were determined from the dry weight (g)



positively co-varied with macrophyte communities that had *N. obtusa* present (Fig. 3). Additionally, concentrations of sodium, potassium, magnesium, calcium, and total nitrogen were positively associated with communities that had *N. obtusa* present (Fig. 3). In contrast, averaged depth, Secchi depth, iron, and TP concentrations were positively associated with macrophyte communities that did not have *N. obtusa* present (Fig. 3). A PERMANOVA confirmed that the macrophyte communities had distinct profiles when *N. obtusa* was present or absent in those communities (Fig. 3) ( $F_{1,47}=4.84$ ,  $p$  value = 0.001).

The gllvm model demonstrated statistically significant ( $p$  value < 0.05) positive co-occurrences between *N. obtusa* and native Characeae such as *Nitella acuminata*, *Nitella mucronata*, *Chara vulgaris*, and *Tolypella intricata* (Fig. 4). Statistically significant ( $p$  value < 0.05) positive associations between *N. obtusa* and other macrophytes were also found including *Eloidea canadensis*, *Potamogeton foliosus*, and *Nuphar lutea* (Fig. 4). In contrast, *N. obtusa* had statistically significant ( $p$  value < 0.05) negative co-occurrence patterns with the majority of other macrophyte taxa in the study lake communities, and most notably with *Myriophyllum spicatum* (Fig. 4).

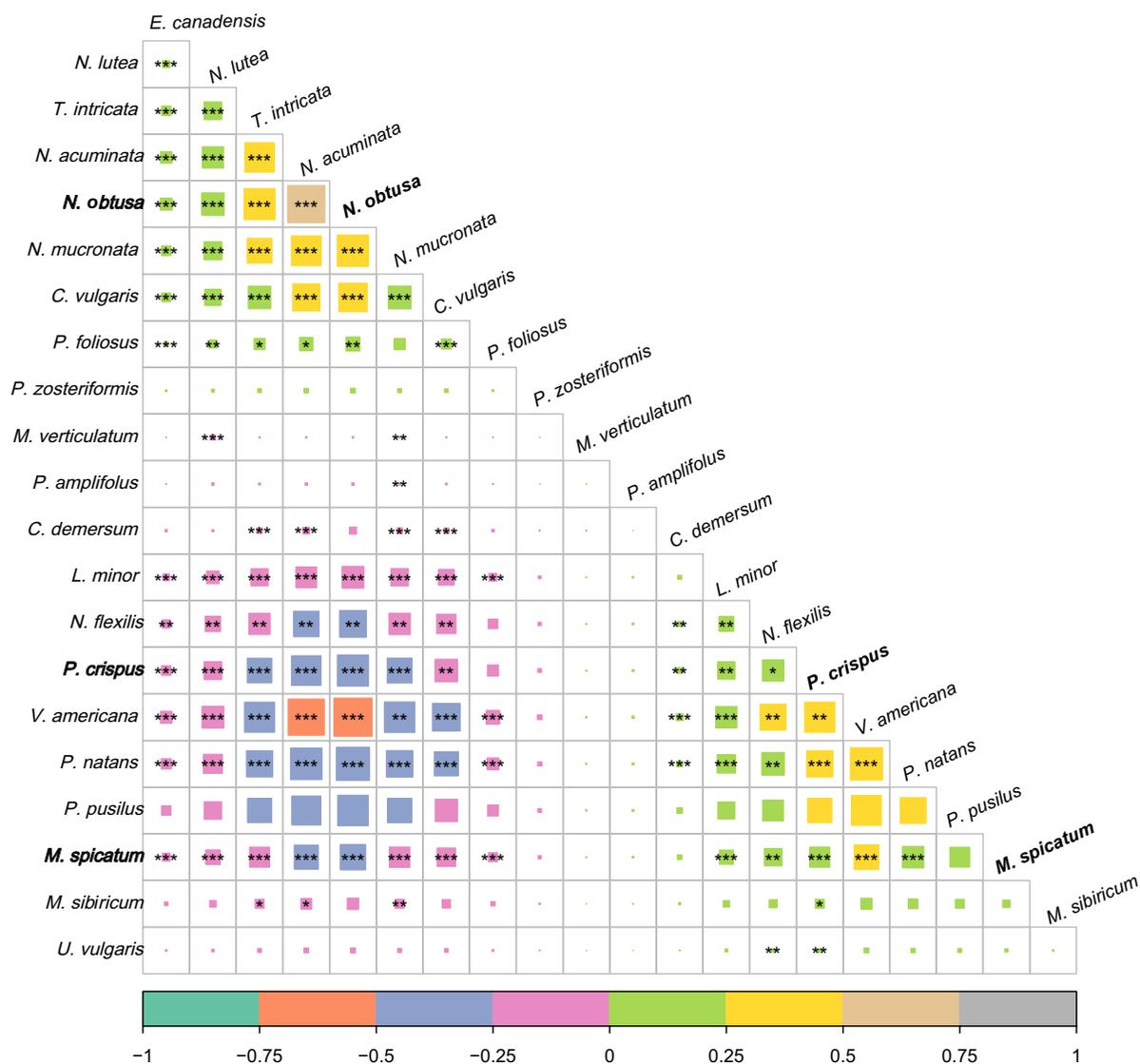
## Discussion

In three of the nineteen study lakes where *N. obtusa* was found, it represented over 80% of macrophyte biomass for the sampled transect. This is the first time that near monoculture dominance of *N. obtusa* in the macrophyte community of several Canadian lakes has been reported. In Ontario, aquatic ecosystems are not systematically monitored for non-native species, until dramatic community-level shifts are observed. Since most of the study lakes, including those with ideal growth conditions (i.e., calcium concentrations > 15 mg · L<sup>-1</sup>) (Harrow-Lyle and Kirkwood 2021a), had no *N. obtusa* or less than 50% relative abundance, it is possible that the *N. obtusa* invasion front in Ontario lakes is still in its early stages. Although increased sampling intensity in each lake may have increased the detection of *N. obtusa* populations, the dataset compiled in this study was robust enough to statistically detect macrophyte community interactions that may be conducive to successful establishment of *N. obtusa* in invaded regions.

Interestingly, the earliest record of *N. obtusa* within North America was first misidentified as a *Chara* spp., which was later corrected through herbarium records (Karol and Sleith 2017). In this study, *N. obtusa* had strong positive co-occurrences with *Chara vulgaris*, *Nitella acuminata*, and *Nitella mucronata*. These species are among the genera that are often mistaken as *N. obtusa*, and are frequently found in tangled masses of *N. obtusa*. This intermingling can thwart identification in the early stages of invasion by lake users and practitioners alike (Pullman and Crawford 2010). Due to common misidentification, it is also plausible that *N. obtusa* has been inhabiting Ontario lakes for decades as sleeper populations. A sleeper population is defined as a non-native species persisting at low abundances until environmental changes have triggered subsequent population increases (Spear et al. 2021).

Generally, when a population explosion of a new non-native species occurs, it is assumed that the introduction occurred recently (Spear et al. 2021). The premise of non-native species persisting within habitats for lengthy periods of time prior to population eruption is often overlooked (Spear et al. 2021). Within our study, we observed 11 instances where *N. obtusa* populations were at low abundance (< 50% relative abundance), often mixed within beds of native Characeae. Given a history of misidentification, it is likely that *N. obtusa* has been present in inland lakes within the Great Lakes basin well beyond the last decade, and was missed due to inadequate macrophyte community monitoring and skilled identification. Populations of *N. obtusa* discovered across the study period all had the presence of the star-shaped bulbils. Typically, bulbils can only be found within established beds, thus we conclude that *N. obtusa* has established populations within our study lakes (Bonis and Grillas 2002). Therefore, future paleolimnological studies may be able to ascertain the time period of initial colonization in invaded lakes via fossilized tissues, including bulbils.

Within *N. obtusa*'s native range, reproduction occurs primarily through vegetative fragments representing asexual and sexual propagules deposited in the sediments (Migula 1897). Sediments can host transient propagule banks that reproduce within one year of deposition (Thompson and Grime 1979), or persistent propagule banks that can last up to 89 years or more (Bonis and Grillas 2002). In contrast, a shift



**Fig. 4** General linear latent variable model (gllvm) output matrix to visualize the species-specific interactions between macrophyte assemblages present within the candidate lakes ( $n=49$ ) when influencing environmental covariates have been accounted for. The gllvm model represents a negative bino-

mial distribution, incorporating influences from five of the water quality parameters; calcium, potassium, pH, manganese, and depth. A heatmap depicting the strength of the correlation between the species covariates is presented. Non-native species encountered across this study have been presented in bold

in reproduction has been observed when Characeae colonize shallow environments (Krause 1985). The development of gametangia and fertility within such environments has been linked to warmer water temperatures, and sunny growing seasons (Willén 1960; Boissezon et al. 2018).

Initial reports surmised that both male and female specimens of *N. obtusa* were present in North

America; however, examination of suspected oogonia revealed that they were all male antheridia (Sleith et al. 2015). Currently, only specimens that do not produce female sexual organs have been documented in North America (Mann et al. 1999; Sleith et al. 2015). Within our study, all samples of *N. obtusa* had distinct, visible male antheridia present. Despite sexual reproduction being typically beneficial in

dynamic shallow environments, as well as facilitating viable long-ranged dispersal, low sexual fertility of *N. obtusa* has been reported as early as the 1800s (Migula 1897).

Though dispersal of *N. obtusa* within invaded regions is not well understood, it is thought to occur through distribution of propagules and vegetative fragments (Reynolds et al. 2015; Green 2016). Propagules are known to spread through epizoochory (Bonis and Grillas 2002); however, there is growing evidence that the bulk of *N. obtusa* transfer is occurring by the movement of watercraft. For example, Midwood et al. (2016) noted that *N. obtusa* was found in areas with higher dock densities, and docks are strongly tied to recreational watercraft activity. Masses of *N. obtusa* found on watercraft and trailers remain viable after several days, suggesting that proper clean and drain protocols are not being followed (Glisson et al. 2019). In addition, we found that populations of *N. obtusa* tended to have the highest biomass at sites nearest boat launches (Harrow-Lyle and Kirkwood 2021a). Given the close proximity of invaded lakes documented in this study to the Trent-Severn Waterway, it is probable that boats serve as an important vector of *N. obtusa* spread. The Trent-Severn Waterway is a lake-canal navigation system that allows free movement of watercraft between Lake Huron and Lake Ontario, and has been implicated as an important conveyance system for invasive species spread (Kelly et al. 2013; Masson et al. 2016).

As more studies are published, it is becoming apparent that *N. obtusa* can alter habitat structure and function, as well as displace other macroalgae and vascular macrophytes (Pullman and Crawford 2010; Brainard and Schulz 2017; Ginn et al. 2021; Harrow-Lyle and Kirkwood 2021b). Total and native macrophyte biomass decreased in response to *N. obtusa* in invaded regions within the United States (Brainard and Schulz 2017). In Ontario, Ginn et al. (2021) also described the displacement of native and invasive macrophytes upon *N. obtusa* establishment within Lake Simcoe. Similarly, the initial identification and subsequent increase in *N. obtusa* within Lake Scugog, Ontario, has resulted in the displacement of native and invasive macrophytes (Harrow-Lyle and Kirkwood 2021c).

In other invaded regions, *Ceratophyllum demersum* and *Utricularia vulgaris* were found to reach nuisance levels in conjunction with *N. obtusa* growth (Pullman

and Crawford 2010). Furthermore, *N. obtusa* has also been found in stands of *C. demersum* within its native range (Pełechaty et al. 2014). In Ontario, our results demonstrate *N. obtusa* negatively co-occurs with these taxa, along with most other members of the macrophyte community. Notably, this study highlights a negative relationship between *Myriophyllum spicatum*, a normally aggressive non-native macrophyte long established in many Ontario lakes over the last 60 years. Within two invaded systems in Ontario, Lake Scugog and Lake Simcoe, similar negative relationships have been observed between these two non-native macrophytes (Ginn et al. 2021; Harrow-Lyle and Kirkwood 2021c).

In our region-wide study across south-central Ontario, we observed a reduction in macrophyte diversity within lakes invaded by *N. obtusa*; however, there was still considerable variation. When examining the species-specific interactions revealed by gllvm, *N. obtusa* had negative co-occurrences with most members of the macrophyte community, but also had significant positive associations with native Characeae across Ontario. Based on these findings, and the possibility that *N. obtusa* is still in its early stages of an invasion front, it is too early to conclude that *N. obtusa* has caused diversity loss and species displacement across Ontario lakes. However, more comprehensive in-depth lake studies such as those done in other invaded systems (Brainard and Schulz 2017; Ginn et al. 2021; Harrow-Lyle and Kirkwood 2021c) do offer supporting evidence of *N. obtusa*'s negative impacts to macrophyte community diversity. To understand this disparity between native and invaded regions requires further study, but it may be due to differences in biotic interactions, such as competitive exclusion, that drives this divergence in environmental tolerance.

Overall, our results provide further evidence that *N. obtusa* threatens ecosystem health and productivity in invaded regions. The displacement of native macrophytes shown in this study will in turn have negative impacts on native fish habitat. With the formation of a physical barrier, reduced nesting area, and spawning activities are likely for fish communities (Pullman and Crawford 2010). Pullman and Crawford (2010) have suggested that fish such as *Ambloplites rupestris*, *Lepomis macrochirus*, and *Lepomis gibbosus* avoid areas with *N. obtusa* growth due to the formation of dense benthic mats. Additionally, other fish

species such as *Sander vitreus*, *Esox niger*, and *Esox masquinongy* have displayed altered hunting strategies, due to the impenetrable *N. obtusa* mat architecture (Pullman and Crawford 2010). Furthermore, we have recently shown that *N. obtusa* can alter the biogeochemical cycling of oxygen and phosphorus with increasing biomass (Harrow-Lyle and Kirkwood 2021b). Finally, *N. obtusa* has been shown to have negative impacts on macroinvertebrate communities (Caraco et al. 1990; Murphy et al. 2018).

In conclusion, *Nitellopsis obtusa* may be the most enigmatic and aggressive, non-native macrophyte species to be introduced into North America. Early identification and mitigation are often foiled when *N. obtusa* is present within stands of native Characeae (Brainard and Schulz 2017). The reduction in macrophyte diversity shown to occur in this study and others, coupled with the frequent misidentification of *N. obtusa*, warrant increased monitoring efforts. Early detection, as with most invasive species, will be critical for lake management efforts aimed at mitigating the negative ecological effects of *N. obtusa* establishment and increasing dominance in inland North American lakes.

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**Author's Contributions** TJHL contributed to the design, data collection, analysis, and writing of the manuscript with support from AEK. AEK supervised the project, validated results, and acquired funding.

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**Data Availability** All data used within this manuscript is available upon the reader's request.

## Declarations

**Conflicts of Interest** We have no conflicting interests to declare.

**Code Availability** All R code used within this manuscript is available upon the reader's request.

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