

## RESEARCH ARTICLE

# Use of native macrophytes for recovery of the habitat structure and complexity of a lowland stream affected by river engineering works: Implications for management

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## Abstract

Macrophytes were transplanted into a lowland stream affected by river engineering works. The aim was to analyse the feasibility of their reintroduction and potential to be used for the recovery of the structure and complexity of the lotic habitat. Macrophytes contribute heterogeneity to streams, modify the current velocity, affect sediment and nutrients dynamics, and provide a substrate for epiphytic biofilm. We transplanted specimens of *Ludwigia peploides*, *Gymnocoronis spilanthoides*, and *Egeria densa* into a stream located in the Pampean plain (Buenos Aires, Argentina). The growth and coverage of the transplanted macrophytes and the changes in the structure of the epiphytic biofilm were assessed. The results show that specimens of *G. spilanthoides* were negatively affected by the transplant and new conditions, *E. densa* decreased its coverage after the transplant, and *L. peploides* endured the transplant and adapted to the dredging site with successful establishment and expansion. There were no significant differences between the biofilm developed in *L. peploides* and *G. spilanthoides*. Additionally, the biofilm had similar features between the transplanted macrophytes and those from a site with no dredging. Considering this result, the selection between these macrophytes in relation to biofilm production is indifferent. However, as *L. peploides* adapts better to the new conditions generated by the river engineering works, its use in the rehabilitation project is recommended. Moreover, it is important to consider the ability of different species of macrophytes to survive the transplant and grow under new environmental conditions in order to include them in rehabilitation projects.

## KEYWORDS

aquatic plants, biofilms, dredging, Pampean streams, restoration

## 1 | INTRODUCTION

River ecosystems are subjected to strong environmental pressures as a consequence of anthropogenic activities, which produce significant changes in the structure and diversity of aquatic communities (Deegan & Ganf, 2008). A usual practice in lowland streams is the implementation of river engineering works such as dredging and channelization, which lead to significant changes to the channel, an increase in

turbidity and in the concentration of solids in suspension, a decrease in light penetration and changes in the concentration of nutrients (Armengol, 1998; Lewis, Weber, Stanley, & Moore, 2001; Licursi & Gómez, 2009).

Significant reductions in the density of benthic invertebrates and losses of species typical of slower-flowing or vegetated reaches have been observed as a consequence of these actions (Cooper et al., 2007; Grygoruk, Frał, & Chmielewski, 2015; Lewis et al., 2001;

Robinson, Newell, Seiderer, & Simpson, 2005; Szlauer-Lukaszewska & Zawal, 2014; Witt, Schroeder, Knust, & Arntz, 2004). Also, the impacts of dredging have been reported for birds (Howarth, Grant, & Hulbert, 1982), fishes (Kjelland, Woodley, Swannack, & Smith, 2015) phytoplankton, periphytic algae (Cabrita, 2014; Lewis et al., 2001; Prat et al., 1999), and diatoms from the epipellic biofilm (Licursi & Gómez, 2009). The loss of the channel and vegetal cover as well as the capacity for retaining propagules have also been documented (Brookes, 1987; Erftemeijer & Lewis, 2006; Riis, 2008). Once the macrophyte community is lost because of human intervention, the probability of regeneration from fragments of plants or seeds (propagules) is too low. Riis, Schultzs, Olsen, and Katborg (2009) found that, in lowland streams, the retention of propagules is 1% of the total of fragments dragged by the downstream current and only 3.4% of this retained fragment achieved successful colonization.

Macrophytes have been described as biological engineers (Sand-Jensen, 1997), as they have the ability to modify the current velocity, affect the sediment and nutrient dynamics, and are highly efficient at removing a variety of contaminants, including heavy metals and organic/inorganic pollutants from the water (Bonanno, Borg, & Di Martino, 2017; Guittouy-Philippe et al., 2015; Salt et al., 1995). Furthermore, they play an important role by providing habitat complexity and structural heterogeneity. They also provide the substrate for the development of the epiphytic biofilm (Vilches, 2012) and are a source of nourishment for different trophic levels (Ocon, Oosterom, Muñoz, & Rodrigues-Capitulo, 2013).

After dredging and rectification practices, the heterogeneity of the channel is considerably reduced in lowland streams. Due to the slight probability for the fragments of plants being retained, it is important to develop techniques to favour the establishment and successful propagation of aquatic plants in these streams.

Plantation and propagation techniques have been developed for the rehabilitation of lakes, where recolonization is sought in order to improve water quality and environmental heterogeneity (Qiu et al., 2001; Zhou et al., 2006; Paice, Chambers, & Robson, 2016). Although these techniques have been tested on lowland streams, they have been hardly studied and their success has been questioned (Delmail et al., 2013; Larned, Suren, Flanagan, Biggs, & Riis, 2006; Riis et al., 2009; Suren, 2009). Due to the fact that aquatic plants constitute a key element of the ecological integrity of these ecosystems, their inclusion in rehabilitation projects is relevant (Riis et al., 2009).

In the Pampean plain, the inappropriate use of the land, inadequate urban planning, and contamination have accelerated the siltation process, leading to the need for dredging and mud extraction activities (Licursi & Gómez, 2009). These practices are carried out in order to deepen, rectify, and widen rivers with the purpose of increasing their discharge capacity, with the consequent loss of aquatic plants and the functions they perform in aquatic ecosystems.

We planted three species of native macrophytes to analyse their growth and survival in the short term in the context of new environmental conditions generated by river engineering works, to evaluate the feasibility of their reintroduction and potential to be used in restoration projects. Additionally, considering the epiphytic biofilm ecological roll as an important source of nourishment for different trophic levels, this research evaluated changes in its structure after

transplantation in order to assess its response to the transplant and the possible differences between plants. The questions were (a) can the species studied be established in a stream affected by river engineering works; (b) which of the established species adapts better to the new conditions; (c) what are the changes in the structure of the epiphytic biofilm developed on this macrophytes after transplantation; and (d) what are the differences in the epiphytic biofilm between plants?

## 2 | METHODS

### 2.1 | Study area

The experiment was conducted in 2015, in the Martín stream, a second-order water course located in the Argentine Pampean plain, flowing into the Río de la Plata estuary (Figure 1), which was affected by river engineering works. The climate is temperate and humid with an annual average temperature of 18 °C and an annual average rainfall of 900 mm (Giorgi, Feijoó, & Tell, 2005). The sediment of the stream is mostly composed of slime-clay with low proportions of gravel and sand. The land uses in the basin are mainly agricultural and peri-urban (Cohero, Licursi, & Gómez, 2015).

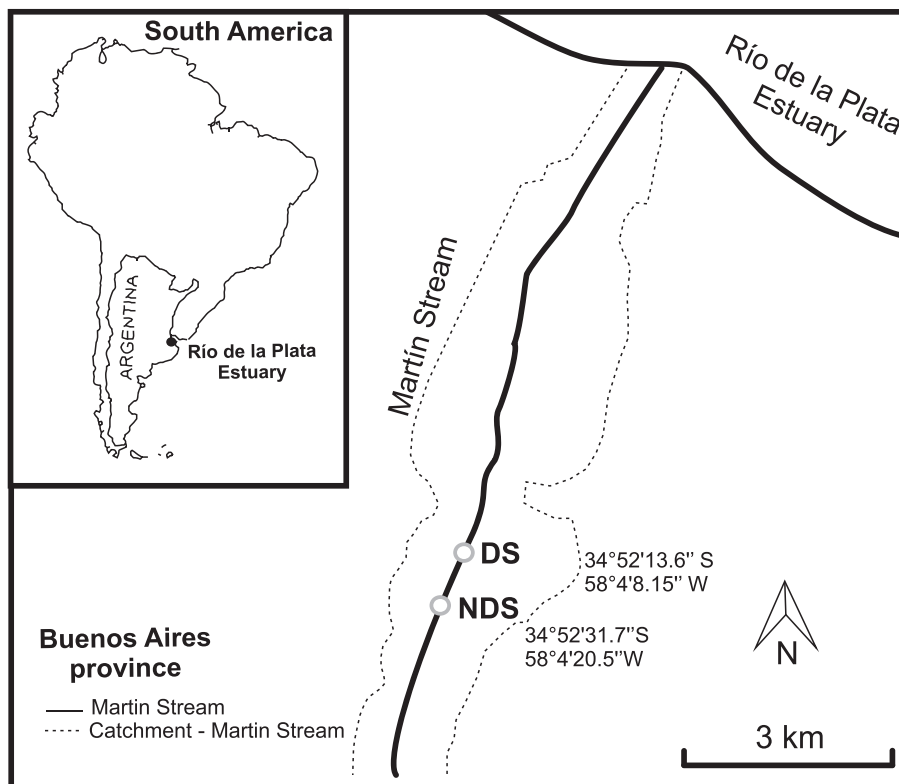
In order to carry out the experiment, two sampling sites were established, a no dredging site (NDS) and a dredging site (DS). The first site was not affected by river engineering works, and it was located 500-m upstream from the second site, at which dredging rectifying and widening works had been carried out involving with the removal of aquatic plants, 15 days previous to the beginning of the experiment. The detail of the morphology of the channel profile in both sites before and after the dredging is shown in Figure 2a,b.

### 2.2 | Experimental setup and planting procedure

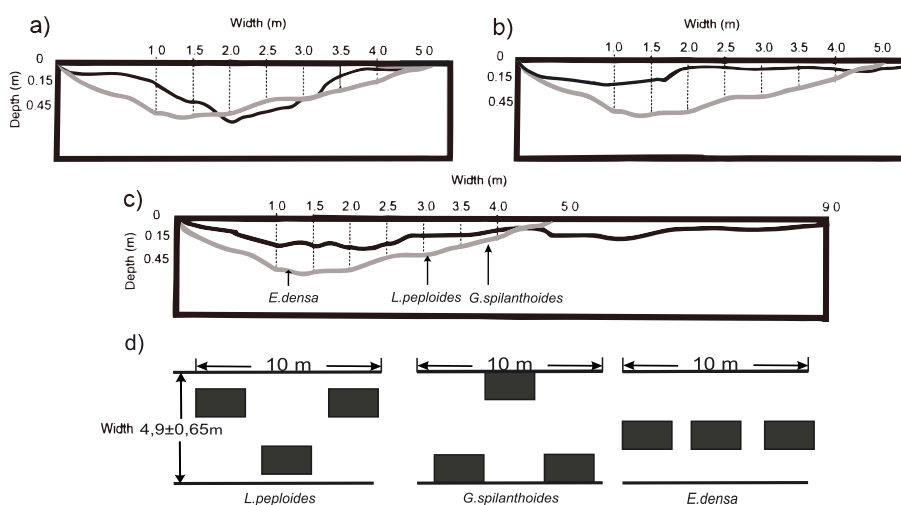
The species selected for the experiment were *Ludwigia peploides* (Kunth) P. H. Raven (Onagraceae), *Gymnocoronis spilanthoides* (Don) DC. (Asteraceae), and *Egeria densa* Planch (Hydrocharitaceae), usually common in lowland stream. *L. peploides* is an herbaceous perennial plant; the life forms of this species are rooted with floating leaves. *G. spilanthoides* is an emergent freshwater or marsh-growing perennial, and *E. densa* is a submerged species that produce roots at intervals along the stem (Cabrera, 1964). These three perennial species are native to South America.

At the NDS, plant beds were set up in trays (45 × 35 × 10 cm) selecting specimens with shoot lengths of 25–30 cm. The collection of specimens was performed manually with a gardening shovel, taking care not to damage the roots and the shoots. In order to exclude the effect of the substrate, sediment from the same stream was used following the recommendation of Riis et al. (2009). Once the plants were established in the trays at the NDS, they were immediately taken to the DS (500-m downstream).

In the DS, each group of nine trays per species were subdivided into three groups to form three beds per species. Trays were buried in the stream at water depths from 0.15 to 0.40 m, depending on the species (Figure 2c) where mean water velocity was between 0.06 and 0.1 m/s. The trays were placed following the recommendations of Bal et al. (2011; Figure 2d).



**FIGURE 1** Map of the study area showing the Martín Stream with the location of the sampling sites; NDS = no dredging site, DS = dredging site



**FIGURE 2** Comparison of channel cross-sections: no dredging site (NDS; grey line) and dredging site (DS; black line); (a) before dredging; (b) 1 month after dredging (start); (c) 3 months after dredging (end); and (d) a plan view of the trays distribution patterns in the dredging site (DS) [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

The purpose of propagating plants in the trays was to allow the plant beds develop an extensive root net and a closed bed shape so that they would be more resistant to disturbance after transplanting into the stream bed. Two samplings were carried out, one 30 days after transplantation (start) and other 90 days after transplantation (end).

### 2.3 | Water quality and hydrogeomorphology

The dissolved oxygen concentration (DO, mg/L), the temperature (°C), the pH, the conductivity ( $\mu\text{S}/\text{cm}$ ), and the turbidity (NTU) were measured in triplicate at each site with a HORIBA Multiparameter U-10. On each

occasion, a water sample was collected, refrigerated, and filtered through 0.45- $\mu\text{m}$  Sartorius membrane filter for the analysis of nutrients. Ammonium, nitrites, and nitrates soluble reactive phosphorous (SRP, mg/L) were analysed according to standard methods (APHA, 1998). The mean depth, stream width, and flow velocity were measured and then the discharge calculation was performed (Elosegi & Sabater, 2009).

### 2.4 | Macrophytes

To evaluate the growth of macrophytes, 10 specimens of each species (at both sites) were marked and the following morphological traits

were measured throughout the experiment: shoot length (distance from shoot base to the shoot apex), internode length (value of internodes located 3-cm below the apex), number of nodes, and lateral shoots (Thiébaud, Gillard, & Deleu, 2016). From the shoot length, we calculated the length increase (LI), taking into account the difference between the lengths of the main shoot at the end of the experiment in relation to the lengths immediately after the transplantation of all specimens (LI = final length of main shoot – initial length; Choudhury, Yang, & Hansson, 2015). To carry out the comparison between species at the same site, the growth rate (RGR) was calculated with the length of the shoots measured (RGR = length growth/time; Vari, 2013).

In order to calculate the coverage of the beds from each species, a series of images were taken using a Nikon 3100 camera on each sampling day following the methodology proposed by Pan, Li, and Sun (2007). These images were then processed using ImageJ Version 1.46 R to quantify the coverage of each bed on all sampling dates, including the size of the bed immediately after transplanting. The increase in coverage was calculated on the basis of the difference between the final and initial cover of each bed at both sites.

## 2.5 | Epiphytic biofilm

In order to analyse the epiphytic biofilm autotrophic fraction (algae) of each plant, a 15-cm fragment (including both stem and leaf) was collected; three of these fragments constituted a replicate with a total collection of three replicates per species, which were refrigerated until arrival to the laboratory. These samples were collected before transplant, at the start and at the end of the experiment.

The epiphytic biofilm was obtained by sonicating samples in an ultrasound bath (Cleanson), for three periods of 2 minutes (Romaní & Sabater, 2001). A fraction of the suspension was used for the quantification of the chlorophyll *a* concentration (Elosegi & Sabater, 2009), and the remaining sample was fixed in 4% formalin. A subsample was used for the density quantification of the algal taxonomic groups and another was used for diatom taxocenosis. For the algae counting, a Sedgewick-Rafter camera and an Olympus BX51 a 200X (APHA, 1998) microscope were used. In order to analyse the diatom chloroplast modifications (shape and size), the methodology of Wood, Mitrovic, and Kefford (2014) was followed. Data are expressed as ind/cm<sup>2</sup> and referred to the surface of the sonicated macrophyte.

For the analysis of the diatom assemblage, the samples were oxidized with hydrogen peroxide and the reagents extracted by successive washing steps by centrifugation. The samples were subsequently mounted in Naphrax®. Four hundred valves from each sample were counted using an Olympus BX51 microscope with interference phase contrast under 1000× magnification. Specific keys were used for taxonomic identification.

## 2.6 | Data analysis

### 2.6.1 | Water quality, hydrogeomorphology, and macrophytes

A *t* test was used for comparisons between the NDS and DS for water quality and hydrogeomorphology in all dates (García-Berthou, Alcaraz Cazorla, Benejam Vidal, & Benito, 2009). A *t* test was performed to

evaluate the differences in the LI of macrophytes between DS and NDS. The number of nodes, lateral branches, and length of each internodes were compared in both sites in all sampling date with a *t* test. A one-way analysis of variance was performed in order to compare the growth rate (RGR) between species in both sites.

### 2.6.2 | Epiphytic biofilm

#### Comparison between macrophytes

Total algal density and the proportion of algal groups between the biofilm developed in *L. peplioides* and *G. spilanthoides* before transplant and at the end of the experiment was tested using a *t* test.

To test whether the diatom assemblage composition in macrophytes differed significantly from each other, an analysis of similarity (ANOSIM) was used. Similarity percentage (SIMPER) was performed to determine to what extent diatom taxa contributed to percentage of dissimilarity between groups (Primer 6.1 software). Both tests were performed before transplant and at the end of the experiment only for DS.

#### Comparison between sites

Differences in total algae density and the proportion of algae groups were compared between sites at both time points (start and end) using a *t* test. Regarding the diatom assemblage, the Shannon–Wiener diversity index ( $H'$ , calculated using  $\ln$ ) was calculated. This index, the richness and the diatom chloroplast modifications, were compared using a *t* test.

To test whether the diatom assemblage composition among plants of each species (between NDS and DS) differed significantly from each other, an ANOSIM was used. SIMPER was performed to determine to what extent diatom taxa contributed to the percentage of dissimilarity between groups (Primer 6.1 software).

## 3 | RESULTS

### 3.1 | Water quality and hydrogeomorphology

Based on the hydrogeomorphologic parameters, the stream width was significantly greater at the DS section during the whole experiment and the depth was significantly lower at the start. These differences were probably because of the works performed at the dredging section. The temperature, the pH, and the dissolved oxygen were significantly higher in the DS than in the NDS at the start and at the end of the experiment (Table 1).

### 3.2 | Macrophytes

Of the three species used, only *E. densa* drastically decreased its coverage after the transplant (<65%) and all specimens marked to evaluate growth were lost. However, the other two species were analysed. The growth rate (RGR) of *G. spilanthoides* was significantly lower (0.299 cm/day ± 0.12) than the RGR of *L. peplioides* (1.69 cm/day ± 0.68) at DS. The same was observed for each species of macrophytes in the NDS (0.79 cm/day ± 0.09 for *G. spilanthoides* and 1.71 cm/day ± 0.75 for *L. peplioides*).

**TABLE 1** Mean  $\pm$  SD of physical, chemical, and geomorphological data at no dredging site (NDS) and dredging site (DS), at the start and at the end of the experiment

|  | Start            |                   | End               |                   |
|--|------------------|-------------------|-------------------|-------------------|
|  | NDS              | DS                | NDS               | DS                |
| Rainfall (mm)                          | 49               |                   | 39                |                   |
| Flow (m/s)                             | 0.086 $\pm$ 0.09 | 0.108 $\pm$ 0.08  | 0.021 $\pm$ 0.014 | 0.023 $\pm$ 0.025 |
| Width (m)                              | 4.16 $\pm$ 0.16  | 4.9 $\pm$ 0.65*   | 4.15 $\pm$ 0.11   | 9.66 $\pm$ 0.57*  |
| Depth (m)                              | 0.18 $\pm$ 0.07* | 0.06 $\pm$ 0.04   | 0.16 $\pm$ 0.10   | 0.14 $\pm$ 0.07   |
| Discharge (m <sup>3</sup> /s)          | 0.05 $\pm$ 0.02  | 0.06 $\pm$ 0.01   | 0.025 $\pm$ 0.011 | 0.023 $\pm$ 0.005 |
| Temperature (°C)                       | 22 $\pm$ 0.27    | 27.62 $\pm$ 0.53* | 18.49 $\pm$ 0.24  | 19.19 $\pm$ 0.36* |
| pH                                     | 6.77 $\pm$ 0.19  | 8.08 $\pm$ 0.13*  | 8.21 $\pm$ 0.2    | 8.25 $\pm$ 0.07*  |
| Conductivity (mS/cm)                   | 0.42 $\pm$ 0.002 | 0.49 $\pm$ 0.08   | 0.95 $\pm$ 0.004  | 0.94 $\pm$ 0.01   |
| Turbidity (NTU)                        | 22.03 $\pm$ 0.28 | 43.63 $\pm$ 27    | 3.23 $\pm$ 1.45   | 2.67 $\pm$ 3.41   |
| Dissolved-oxygen (mg/L)                | 4.19 $\pm$ 0.36  | 8.25 $\pm$ 0.74*  | 4.14 $\pm$ 2.82   | 8.41 $\pm$ 1.37*  |
| P-PO <sub>4</sub> <sup>3-</sup> (mg/L) | 0.67             | 0.57              | 0.96              | 0.65              |
| N-NO <sub>3</sub> <sup>-</sup> (mg/L)  | 0.13             | 0.19              | 1.03              | 0.91              |
| N-NO <sub>2</sub> <sup>-</sup> (mg/L)  | 0.11             | 0.12              | 0.23              | 0.1               |
| N-NH <sub>4</sub> <sup>+</sup> (mg/L)  | 0.4              | 0.23              | 1.1               | 0.34              |

Note. Asterisks indicate significant differences between NDS and DS. NDS = no dredging site; DS = dredging site.

\* $p < .05$ .

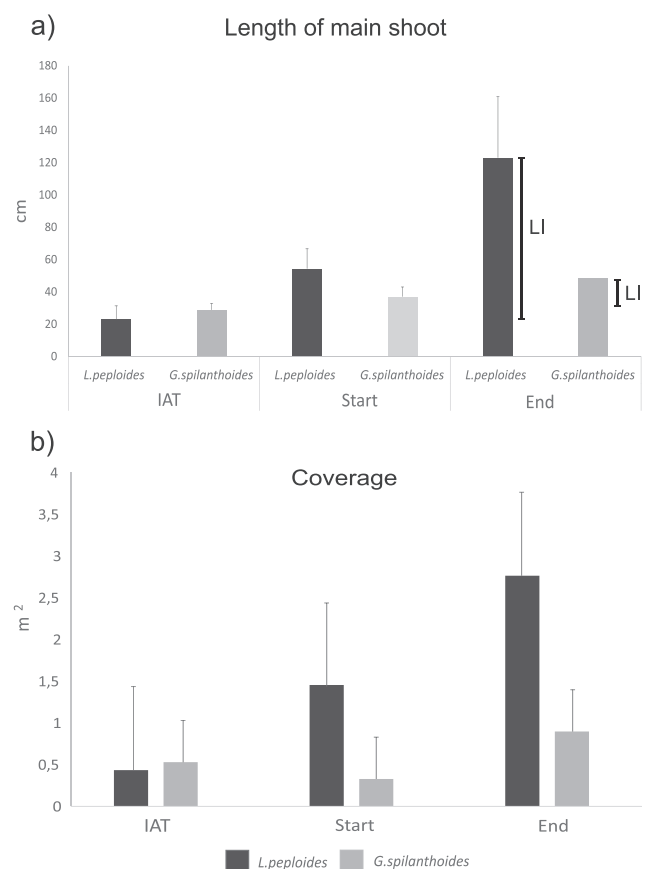
The LI of *G. spilanthisoides* in DS was significantly lower than in the NDS (19.4  $\pm$  6.9 and 45.5  $\pm$  5.2 cm, respectively,  $p = .01$ ). No differences were observed in the LI of *L. peploides* between sites (100.1  $\pm$  40.2 cm at DS and 1009.9  $\pm$  44.4 cm at NDS; Figure 3a). Regarding the number of nodes, the length of the internodes and the number of lateral branches, significant differences were found only in the number of nodes of *G. spilanthisoides* between DS and NDS at the end of the experiment ( $p < .001$ ; Table 2). The coverage of *G. spilanthisoides* was significantly lower in the DS at the end of experiment ( $p = .006$ ), whereas no differences were found in *L. peploides* for the same date (Figure 3b).

### 3.3 | Epiphytic biofilm

#### 3.3.1 | Comparison between macrophytes

The comparison between the biofilm developed in *L. peploides* and the developed in *G. spilanthisoides* before transplant did not show significant differences in the total densities or in the proportion of algal groups. The same results for the total densities and the proportion of algal groups were observed at the DS at the end of the experiment.

The diatom assemblages developed in *L. peploides* and *G. spilanthisoides* before transplant were significantly different (ANOSIM,  $R = .479$ ,  $p = .029$ ). The percentage of dissimilarity was 22.09%, and the species that contributed the most were *Nitzschia amphibia*, *Nitzschia cf. dealpina*, and *Nitzschia desertorum*. Regarding the epiphytic diatom assemblages between plants, at the end of the experiment at the DS, the ANOSIM showed that there were significant differences between assemblages (Global  $R = .46$ ,  $p = .029$ ). Based on SIMPER analysis results, the dissimilarity was of 37.8%, and the species that contributed most were *Cocconeis placentula*, *Nitzschia palea*, and *Navicula veneta*.



**FIGURE 3** Two of the variables of growth measured in both macrophytes at dredging site. (a) the average and SD of the length of the main shoots immediately after transplant (IAT) and at the end of the experiment (end). The line on the right of the bars is the length increase (LI) = final length of main shoot – initial length. (b) the average cover values and SD measured immediately after transplant (IAT), a month later (start) and at the end of the experiment (end)

**TABLE 2** Mean  $\pm$  SD of variable of growth measured during the experiment in *Ludwigia peploides* and *Gymnocoronis spilanthoides*

|                           | <i>L. peploides</i> |               |                |                 | <i>G. spilanthoides</i> |               |                 |                |
|---------------------------|---------------------|---------------|----------------|-----------------|-------------------------|---------------|-----------------|----------------|
|                           | Start               |               | End            |                 | Start                   |               | End             |                |
|                           | NDS                 | DS            | NDS            | DS              | NDS                     | DS            | NDS             | DS             |
| Nodes (number)            | 8.4 $\pm$ 2.3       | 7 $\pm$ 1.4   | 24.1 $\pm$ 4.3 | 21.4 $\pm$ 3.43 | 3.75 $\pm$ 0.5          | 5.5 $\pm$ 0.6 | 11.5 $\pm$ 0.5* | 7 $\pm$ 0.8    |
| Length of internodes (cm) | 2.5 $\pm$ 0.5       | 3.7 $\pm$ 1.2 | 5.8 $\pm$ 0.9  | 6 $\pm$ 0.6     | 4.9 $\pm$ 0.6           | 5.9 $\pm$ 1.8 | 5.7 $\pm$ 0.8   | 6.2 $\pm$ 0.83 |
| Lateral branches (number) | 0                   | 0.6 $\pm$ 0.5 | 14.4 $\pm$ 6.7 | 13.2 $\pm$ 7.5  | 1 $\pm$ 0.8             | 1 $\pm$ 0     | 4 $\pm$ 1.4     | 3.25 $\pm$ 0.9 |

Note. Asterisks indicate significant differences between NDS and DS. NDS = no dredging site; DS = dredging site.

\* $p < .05$ .

**TABLE 3** Mean  $\pm$  SD of epiphytic biofilm variables measured in *Ludwigia peploides*

|   | <i>Ludwigia peploides</i> |                          |                       |                       |
|---|---------------------------|--------------------------|-----------------------|-----------------------|
|   | Start                     |                          | End                   |                       |
|   | NDS                       | DS                       | NDS                   | DS                    |
| Diatoms (cel/cm <sup>2</sup> )  | 54,435.1 $\pm$ 32,541.5   | 85,780.4 $\pm$ 13,689.7  | 5,258.4 $\pm$ 2,854.3 | 4,286.2 $\pm$ 1,828   |
| Chlorophytes (cel/cm <sup>2</sup> )   | 10,772.8 $\pm$ 7,105.5    | 3,396 $\pm$ 1191.7       | 1,487.3 $\pm$ 688.2   | 363.5 $\pm$ 129.5     |
| Cyanophytes (cel/cm <sup>2</sup> )  | 86,574.6 $\pm$ 77,369.5   | 75,373 $\pm$ 13,247.8    | 849.7 $\pm$ 538.4     | 1,547.5 $\pm$ 672.8   |
| Density of autotrophs (cel/cm <sup>2</sup> )                                | 151,782.6 $\pm$ 116,111.6 | 164,550.4 $\pm$ 25,561.8 | 7,595.4 $\pm$ 4,032.1 | 6,197.2 $\pm$ 2,574.9 |
| Chlorophyll <i>a</i> ( $\mu$ g/cm <sup>2</sup> )                            | 0.7 $\pm$ 0.3             | 0.6 $\pm$ 0.4            | 0.2 $\pm$ 0.1         | 0.1 $\pm$ 0.03        |
| Alteration in chloroplast morphology (shape and size; cel/cm <sup>2</sup> ) | 7,164.6 $\pm$ 5,172.4     | 11,289.5 $\pm$ 1728.9    | 654.6 $\pm$ 361.5     | 659.7 $\pm$ 276.8     |
| Species number  | 33 $\pm$ 1.7*             | 18.6 $\pm$ 3.05          | 28.7 $\pm$ 2.9        | 26.3 $\pm$ 1.1        |
| Shannon–Wiener index (bits/ind)   | 3.4 $\pm$ 0.1*            | 2.4 $\pm$ 0.3            | 2.9 $\pm$ 0.5         | 3.5 $\pm$ 0.1         |

Note. Asterisks indicate significant differences between NDS and DS. NDS = no dredging site; DS = dredging site.

\* $p < .05$ .

**TABLE 4** Mean  $\pm$  SD of epiphytic biofilm variables measured in *Gymnocoronis spilanthoides*

|   | <i>Gymnocoronis spilanthoides</i> |                          |                       |                       |
|---|-----------------------------------|--------------------------|-----------------------|-----------------------|
|   | Start                             |                          | End                   |                       |
|   | NDS                               | DS                       | NDS                   | DS                    |
| Diatoms (cel/cm <sup>2</sup> )  | 48,445.8 $\pm$ 16,557.9           | 86,337.7 $\pm$ 21,839.8  | 3,724.4 $\pm$ 2,651.1 | 1,023.1 $\pm$ 672.2   |
| Chlorophytes (cel/cm <sup>2</sup> )   | 5,319.9 $\pm$ 3,408.2             | 1,199.8 $\pm$ 441.1      | 1,046 $\pm$ 618.1     | 1,005.2 $\pm$ 691.3   |
| Cyanophytes (cel/cm <sup>2</sup> )  | 62,641.3 $\pm$ 18,759.8           | 42,457.9 $\pm$ 10,978.9  | 3,190.6 $\pm$ 3,789.6 | 1,174.4 $\pm$ 913.1   |
| Density of autotrophs (cel/cm <sup>2</sup> )                                | 116,407.1 $\pm$ 28,993.9          | 129,995.5 $\pm$ 33,032.5 | 7,961.0 $\pm$ 6,882.8 | 3,202.8 $\pm$ 2,228.3 |
| Chlorophylla( $\mu$ g/cm <sup>2</sup> )                                     | 1.1 $\pm$ 0.5                     | 0.7 $\pm$ 0.3            | 0.1 $\pm$ 0.04        | 0.05 $\pm$ 0.02       |
| Alteration in chloroplast morphology (shape and size)(cel/cm <sup>2</sup> ) | 3,815.1 $\pm$ 3,461.7             | 25,056.9 $\pm$ 16,292.6  | 498.4 $\pm$ 321.1     | 169.5 $\pm$ 20.8      |
| Species number  | 32.7 $\pm$ 1.1*                   | 21.7 $\pm$ 3.5           | 26.7 $\pm$ 3          | 25 $\pm$ 2.6          |
| Shannon–Wiener index (bits/ind)   | 3.4 $\pm$ 0.03*                   | 2.3 $\pm$ 0.1            | 3.1 $\pm$ 0.3         | 3.3 $\pm$ 0.3         |

Note. Asterisks indicate significant differences between NDS and DS. NDS = no dredging site; DS = dredging site.

\* $p < .05$ .

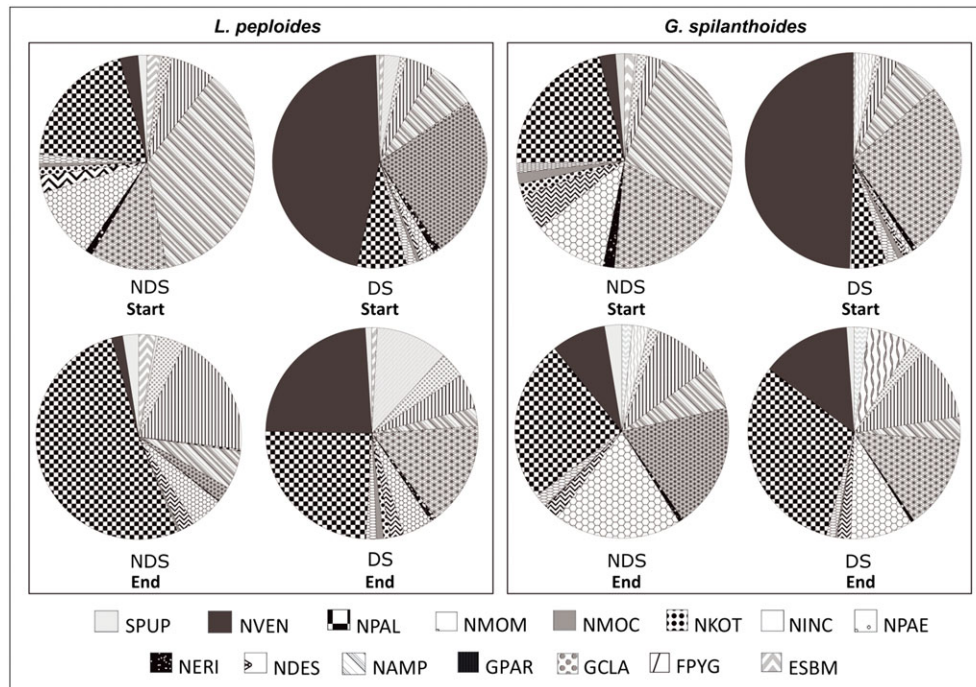
### 3.3.2 | Comparison between sites

The total algal density found in the biofilm of both plants did not show significant differences between the NDS and DS at both time points (Tables 3 and 4).

The diatom assemblage analysis allowed the identification of 61 diatom species in *L. peploides* and 59 species in *G. spilanthoides*; out of the total identified species, 52 were common to both species (see Table S1). At the beginning of the experiment (start), the richness (*L. peploides*:  $p = .002$ ; *G. spilanthoides*:  $p = .007$ ) and the Shannon–Wiener index (*L. peploides*:  $p = .009$ ; *G. spilanthoides*:  $p = .001$ ) were

significantly lower in DS for both macrophytes than in NDS. On the other hand, no significant differences were observed in the chloroplast morphology of the diatoms found in the biofilm of both macrophytes between sites (Tables 3 and 4).

The ANOSIM performed to test for differences in the epiphytic diatom assemblages that developed on *L. peploides* between sites showed that there were significant differences (Global  $R = .856$ ,  $p = .002$ ). The SIMPER analysis showed, at the beginning of the experiment, a dissimilarity of 69%, and the species that contributed most were *N. veneta*, *N. desertorum*, and *N. amphibia*. At the end of the



**FIGURE 4** Relative abundances of the most frequent diatom species: *Eolimna subminuscula* (ESBM); *Fallacia pygmaea* (FPYG); *Gomphonema clavatum* (GCLA); *Gomphonema parvulum* (GPAR); *Nitzschia amphibia* (NAMP); *Navicula cf dealpina* (NDEA); *Nitzschia desertorum* (NDES); *Navicula erifuga* (NERI); *Nitzschia paleacea* (NPAE); *Nitzschia inconspicua* (NINC); *Luticola kotschyi* (LKOT); *Navicula monoculata* (NMOC); *Navicula monoculata* var. *omissa* (NMOM); *Nitzschia palea* (NPAL); *Navicula veneta* (NVEN); *Sellaphora pupula* (SPUP) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

experiment, the dissimilarity between sites was 61% and the species that contributed the most to this dissimilarity were *N. palea*, *C. placentula*, and *N. veneta* (Figure 4).

In the case of *G. spilanthoides*, the ANOSIM also showed that there were significant differences in the epiphytic diatom assemblages between sites (Global  $R = .398$ ,  $p = .028$ ). The SIMPER analysis showed, at the beginning of the experiment, a dissimilarity of 68% and the species that contributed most were *N. veneta*, *N. cf. dealpina*, and *N. desertorum*. At the end of the experiment, the dissimilarity between sites was 46% and the species that contributed the most to this dissimilarity were *N. desertorum*, *Nitzschia paleacea*, and *N. palea* (Figure 4).

## 4 | DISCUSSION

During this study, a macrophyte transplant trial was performed with the purpose of analysing the feasibility of the use of native aquatic plant species for the recovery of habitat structure and the complexity of a lowland stream disturbed by river engineering works. Our results show that this technique allows for the recolonization of macrophytes after dredging and channelization, and this could be useful for management.

The physical and chemical parameters of the Martín stream are characteristic for a Pampean stream (Feijoó & Lombardo, 2007) and were within the optimal values for growth of the three species studied here; nevertheless, the three species responded differently to the new site.

In the case of *L. peploides*, Rejmánková (1992) found that this species showed an increase in dry weight between 20°C and 35°C, in agreement with Yen and Myerscough (1989). Moreover, the high concentrations of nutrients observed at both sites of the stream could have favoured the growth of this species. Gérard, Brion, and Triest (2014) showed an increase in both biomass and length in water with an overall phosphorus concentration of 0.39 mg/P.L. In this study, the establishment of *L. peploides* at the DS was successful, reflected in the increased length of the shoots at this site as well as in the specimens in the NDS; the others descriptors analysed (the number of nodes, length of the internodes, and number of lateral branches) showed the same response. These are important indicators of how *L. peploides* adapted to the new site. The facility of *L. peploides* to grow and survive in a short period of time and in the conditions generated by river engineering works could be related to the presence of high levels of polymorphism and to the phenotypic plasticity of this species (Dandelot, Verlaque, Dutartre, & Cazaubon, 2005), demonstrating its ability to adjust to transplant.

Although there were favourable conditions for the growth of *G. spilanthoides* at both sites, that is, optimal values of temperature and nutrients (Ardenghi, Barcheri, Ballerini, & Cauzzi, 2016; Timmins & Mackenzie, 1995), we found differences in this variable between sites. The difference in the length of the shoots could be related to the unchanging number of nodes in the specimens in the DS throughout the experiment, resulting in a lower shoot length in comparison with those in the NDS. Regarding coverage, differences were also found. Therefore, the reduced growth after

transplanting demonstrates that these specimens were negatively affected.

In spite of the drastic reduction in coverage recorded at the DS for *E. densa* during the experiment, other authors have found that this species has a high capacity of regeneration and colonization (Santos, Anderson, & Ustin, 2011; Vari, 2013). However, like other species, it is negatively affected by some environmental factors such as fluctuations of the water level. Feijoó, Momo, Bonetto, and Tur (1996) and Carrillo et al. (2006) reported that *E. densa* does not develop at depths less than 50 cm, although at the NDS, it was found at a depth of approximately 30 cm. For that reason, the specimens transplanted to the DS were planted at similar depths. Nevertheless, during the first month of the experiment, there was a drought, attributable to low rains and river engineering works. Drawdowns have effects on plant diversity because different species tolerate different water levels (Keddy & Fraser, 2000; Riis & Hawes, 2002), due to the intrinsic ability of species to tolerate dewatering (Bunn & Arthington, 2002; Holmes, 1999). Dugdale, Clements, Hunt, and Butler (2012) found that exposure of *E. densa* to desiccation decreases the amount of plant material. In our study, the decrease in the water level resulted in the exposure of parts of the *E. densa* beds to desiccation; this change in the water level did not affect the other two species. In the case of *L. peploides*, it has an aptitude to grow under different water regimes from a depth of 1–50 cm, which allows this species to survive a drought and colonize new environments (Hussner, 2010; Rejmánková, 1992; Yen & Myerscough, 1989). With regards to *G. spilanthoides*, its life form allows it to survive in shallow water as well (Timmins & Mackenzie, 1995).

To date, only Timmins and Mackenzie (1995) have reported a growth rate of 15 cm per week for *G. spilanthoides*. This result is 3 times higher than ours, but these authors did not specify their conditions or the methodology used. Conversely, the growth rate observed for *L. peploides* was similar to those reported by other authors (Rejmánková, 1992; Hussner, 2010). The different growth rates found in this study reflect the differences in growth form, morphology, and physiology among the species (Nielsen & Sand-Jensen, 1991). *L. peploides* had a higher RGR that was not affected by the transplant technique; as a consequence, we found the same RGR at both sites.

Studies that have used the transplant technique in lowland streams have provided different results, depending on the species and the conditions. Riis et al. (2009) transplanted specimens of *Ranunculus* (Ranunculaceae), *Callitriche* (Plantaginaceae), *Potamogeton perfoliatus* L., *Potamogeton pectinatus* L. (accepted name: *Stuckenia pectinata* (L.) Börner), *Potamogeton crispus* L. (Potamogetonaceae), and *Myriophyllum spicatum* L. (Haloragaceae) and found that they all survived, with the exception of *Callitriche*. Suren (2009) used *Myriophyllum triphyllum* and lost the transplanted material because of harsh hydraulic conditions during the study. However, no studies have assessed the responses of the epiphytic biofilm and macrophytes in the same experiment, despite the fact that they are fundamental components of the food web that can influence higher trophic levels (Ocon et al., 2013).

Regarding the epiphytic biofilm, the results of richness and Shannon diversity of the diatom assemblage in both plants 1 month

later after transplant are in agreement with those presented by Bona et al. (2008). They found that, in rivers that present physical structural modifications, the Shannon–Wiener index of the diatom assemblage was significantly lower. These results were also in accordance with those of Maddock (1999) and Boon (1992). The changes observed in the taxocenosis of the diatoms are coincident with those reported by Licursi and Gómez (2009) in Pampean plain watercourses subjected to dredging. Those authors reported changes in the light climate and the nutrient concentration as a result of dredging that were accompanied by changes in the species proportion of the diatom assemblage in the epipellic biofilm. In our study, similar changes were observed in the diatom assemblage of the epiphytic biofilm.

Considering the results of this study, the biofilm developed in both plants had similar characteristics regarding the density and proportion of algal groups. In this way, both macrophytes provide similar resources to invertebrates. Therefore, the responses in the biofilm after transplant made the selection between plants indifferent. In spite of the diverse benefits that submerged macrophytes contribute to streams (Abu Bakar, Yusoff, Fatt, Othman, & Ashraf, 2013; Ferreira, Giorgi, & Feijoó, 2014; Mazzeo et al., 2003; Yarrow et al., 2009), the use of *E. densa* in the rehabilitation of urban and lowland stream projects is not recommended because of frequent hydrologic fluctuations (Konrad & Booth, 2005). This species might be a good option in streams with more stable water levels. However, further studies with different submerged species of macrophytes are necessary to determine their successful in restoration projects.

*L. peploides* endured the transplant and quickly adapted to the new conditions as a consequence of river engineering works, with successful establishment and expansion in the affected area. This species is used in artificial wetlands because of its capacity to absorb nutrients (Deaver, Moore, Cooper, & Knight, 2005) and due to its complex structure, which provides refuge and food to macroinvertebrates (Stewart, Shumaker, & Radzio, 2003; López-van Oosterom et al., 2016). Thus, the use of *L. peploides* in the rehabilitation of urban and lowland stream projects is highly recommended.

We conclude that it is important to take into account the ability of different species of macrophytes to survive the transplant and to grow under the new environmental conditions. This study has provided us with a better understanding of the community response and which macrophytes should be included in lowland stream rehabilitation projects.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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