Wastewater nitrogen is linked to changes in submerged aquatic vegetation and invertebrates in Oyster Pond

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Abstract

In order to assess the affect of wastewater nitrogen on submerged aquatic vegetation (SAV) and its associated invertebrates we took six random core samples from established stands in each of six recharge zones in Oyster Pond, a coastal estuary in Cape Cod, Massachusetts. We measured the biomass (g m^{-2}) of the plants and abundance (# m^{-2}) of invertebrates, as well as the $\delta^{15}N$ values of plant tissues. The dominant plant *Ceratophyllum demersum* showed an increased $\delta^{15}N$ signature in areas with higher rates of wastewater N loading. *C. demersum* and other plant species changed their contribution to total biomass with higher wastewater N input, with *C. demersum* comprising up to 80% in the areas of highest N loading. Midges and phantom midge larvae increased in abundance with increasing wastewater input and this was not a function of increasing plant biomass. Sensitive taxa, mayflies and caddisflies, had higher abundances in the macrophyte *Chara vulgaris* and not *C. demersum*. We estimate that *C. demersum*, found at depths of up to 3 m could be holding 32% of the annual N load of Oyster Pond.

Introduction

Submerged aquatic vegetation (SAV) has an important structuring role in the dynamics of shallow eutrophic lakes. Aquatic macrophytes may out-compete phytoplankton (a major component of turbidity) for nutrients directly, as well producing allelopathic substances that affect phytoplankton growth (Sheffer et. al. 1993). Additionally, SAV canopies create suitable conditions for denitrification, and supply shelter for zooplankton which may graze on phytoplankton (van Donk and van de Bund. 2002).

Oyster Pond is a shallow, estuarine kettle pond in the southwestern portion of Cape Cod, Massachusetts. In recent years, local residents have become concerned because of increases in

SAV, which have become a nuisance to boaters. Over the last year alone the standing crop of SAV has increased (Sara Grady, personal comm.).

Increased nutrient loads may be one change promoting blooms of SAV. In the case of Oyster Pond, land derived nitrogen loads (S. Good, this volume) in 2002 are more than 10 times the amount that entered the Pond in the eighteenth century (Emery, 1997). Wastewater nitrogen from septic tanks now comprises between 60% and 80% of the nitrogen loading into the pond (S. Good, this volume). To understand the role of SAV in the pond it is important to investigate the connection between nutrient loading and submerged vegetation.

There are a variety of methods to examine this connection. Stable isotope analysis reliably tracks nitrogen from a given external source (Robinson, 2001). Watershed derived nitrogen that enters estuaries typically has high $\delta^{15}N$ values (McClelland and Valiela 1998), and this signature may be detected in the tissues of any organism which takes up this nitrogen.

Other variables may also demonstrate a wastewater nitrogen linkage to the SAV. The biomass of a single species of submerged macrophyte may not be a reliable indicator of increased nitrogen input (Best 1979), but recent studies have shown that nitrogen input can affect the proportion that different species contribute to total biomass (Engelhardt & Ritchie 2001; Lehmann & Lachavanne 1999). It is also important to know the amount of nitrogen held in the total biomass of SAV to understand its importance in the nitrogen cycling of the Pond.

The SAV canopies proliferating in Oyster Pond also harbor a suite of freshwater macroinvertebrates that may also be sensitive to N loads. Macroinvertebrates such as mayflies and caddisflies are affected by nutrient influx (Scarsbrook et al. 2000). Aquatic communities with more mayfly nymphs can shift to those with more midge larvae with higher nitrogen input (Lenat & Crawford 1994). These shifts in fauna that may be associated with plant biomass changes could then extend their influence, for example by altering the cover and food supply for consumers such as fish.

The goal of this study is to examine the effect of total N and wastewater loading on the biomass of submerged macrophytes in Oyster Pond, as well as on the invertebrates that they harbor. First we used $\delta^{15}N$ signatures for SAV to explicitly link wastewater nitrogen load to the organisms in the pond. Second, we sampled SAV biomass to examine the relationships to different nitrogen loads entering different areas of Oyster Pond. Third, we evaluated the relationship of invertebrate species to wastewater nitrogen loading as well as to the biomass of different species of SAV.

Materials and Methods

Stable Isotope Analysis

We took samples of one or two representative plant species from across each recharge zone for isotope analysis. The samples were washed with distilled water, and dried for 24 hours at 60°C. We then ground each sample to a powder with a mortar and pestle and weighed out 5 mg to be sent to the University of California at Davis Stable Isotopes Facility to be analyzed on a mass spectrometer.

Macrophyte Sampling

To ascertain the effect of nitrogen loading on plant biomass we first chose sampling sites from around Oyster Pond that receive N loads from each of six recharge zones that had been delineated by Sarah Good (this volume), based on water table contours (Fig. 1). The N loads vary from 7 kg yr^{-1} in zone 7 to 340 kg yr^{-1} in zone 3 (table 1). In each zone, we took 6 samples from established stands of vegetation at depths of less than 2 meters. We used a plastic cylinder

(26 cm diameter) as a coring device; an attached 0.5 mm plankton net retained all macrophytes and invertebrates.

Plants were sorted to species, blotted dry, and weighed. An aliquot of each species was dried for 24 hours at 60°C and weighed to obtain dry weight.

Invertebrate Abundance

We sieved the plant samples for macroinvertebrates over a 1mm sieve, and sorted the invertebrates to order or family and counted them.

Results and Discussion

We identified 6 genera of submerged aquatic vegetation in Oyster Pond (Table 1). *Ceratophyllum demersum* (Coontail) was the dominant plant species by biomass (g dry weight m^{-2}) in all recharge zones except for zone 2. *C. demersum* formed dense monotypic stands at depths between 2 and 3 meters, but was mixed with other species, particularly *Najas flexilis* (Bushy Pondweed), above 2 m (the littoral zone).

Stable Isotope Analysis

$\delta^{15}N$ values in floating plants (Table 2) increased significantly as wastewater nitrogen load increased (Fig. 2). Floating plants take up nutrients from the water and are likely to reflect the signature of the N in the water. Submerged plants that have well developed roots showed no relationship to wastewater N (Fig. 2). Rooted plants derive most of their nutrients from the substrate (where constant recycling of N leads to higher $\delta^{15}N$ values) while floating plants must take up nutrients from the surrounding water. Floating plants such as *C. demersum* could be used to monitor wastewater input by measuring their $\delta^{15}N$ values.

Macrophytes

We found no correlation between nitrogen loading from wastewater and mean plant biomass, or the mean biomass of the dominant plant, *C. demersum* (Fig. 3). Individual species therefore showed no evident response to N loads. If, however, we considered the assemblage of major plant species (Fig. 4), there were notable shifts in the species that contributed biomass to the macrophyte canopies. The average proportion of *C. demersum* biomass in the littoral zone increased significantly with increasing wastewater nitrogen load ($F = 16.68$, $p < 0.05$), while the proportion of both *Najas flexilis* and of *Potamogeton* biomass correspondingly decreased (Fig. 4). Similar shifts in plants species composition have been reported elsewhere (Chambers and Prepas 1990; Lehmann & Lachavanne, 1999).

Using a bathymetry map of Oyster Pond (Emery, 1997) in Arcview 8.2, we calculated the total area of each recharge zone that is shallower than 3 m (the deepest extent to which we observed *C. demersum* growing)(Fig.1). Using this value and the percent nitrogen in *C. demersum* tissue (derived from our stable isotope analysis), we estimated that throughout the pond, *C. demersum* is storing about 279 kg of N.

The increase in aquatic plants in Oyster Pond may have considerable effect on water quality of the Pond. Plant surveys done in 2001 (Grady and Muto 2001) showed much less developed plant canopies. In one year then, perhaps 279 kg of N may have become stored in plant tissues. This one year increase is comparable to 32 % of the annual N load received by Oyster Pond from its watershed (S. Good, this volume). This suggests that the plants are holding a considerable part of the annual N input. Moreover, our data from 2002 show a 37% decrease in dissolved inorganic nitrogen ($NO_3 + NH_4$) (Dixon et al., this volume) compared to similar measurements done in 2001 (Al-Qatami et al. 2001). It may be that the decrease is related to the uptake and storage of DIN by plants. This implies that the aquatic plants, while a nuisance to boaters, may be also improving water quality. If the plants were not there, the nutrient concentrations in the water would be substantially higher. This, in addition, suggests that a management option might be to rake floating aquatic plants out of the pond and export the biomass away from the pond, perhaps for use as green manure or compost elsewhere.

Invertebrates

Our sampling collected 17 different groups of invertebrates with large ranges in abundance among replicates. The representative taxa at each site were similar, so to test invertebrate variables with wastewater N load, and macrophytes of Oyster Pond, all 33 replicates were combined.

The abundance of midges and phantom midges increased in the areas receiving higher N load (Fig. 5). Midges are an important food for fish, but unmanaged populations can cause increases in the swarms of the flying adult form. The midge abundance did not increase with increasing plant biomass, but seemed to reach a peak at intermediate biomass (200 g m-2), before lowering at the highest biomasses (Fig. 6). The pattern, although not statistically analyzed, was similar for midges at the sites of the highest and lowest wastewater N loading. More detailed sampling is necessary, but this suggests a preferred level of plant biomass by the midges.

Samples with a higher proportion of *Chara vulgaris* had higher abundances of mayfly nymphs and caddisfly larvae (Fig. 7). Charophytes have a strong ability to compete with phytoplankton and reduce turbidity (van Donk and van de Bund. 2002, Blindow 2002). It could be that *Chara vulgaris* is providing less turbid waters for these sensitive taxa (Harding et. al., 1999). There was also an increase in overall number of invertebrate taxa in samples that had a higher percentage of *Chara vulgaris* (Fig. 7).

In conclusion, changes in the species composition of SAV and its associated invertebrates in Oyster Pond can be used to infer the wastewater nitrogen load in a given area of Oyster Pond. Additionally, the large biomass of *C. demersum* this year has sponged up about one-third of the annual nitrogen load from the Oyster Pond watershed. In the future it will be important to account for this factor when making management decisions about the Pond.

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Table 1. Mean ± SD for plant biomass (dry weight m^{-2}) in the 6 recharge zones in Oyster Pond. The sign (+) stands for trace amounts

	Recharge zone					
	1	**2**	**3**	**4**	**5**	**7**
Ceratophyllum demursum	79±54	0	146±103	169±161	104±181	96±151
Najas flexilis	67±87	30±59	29±41	172±204	88±115	0
Potamogeton spp.	2±3	10±16	0	0	0	0
Chara aspera	0	37±48	0	0	0	11±27
Ruppia maritima	0	0	0	0	0	58±65
Lemna minor	(+)	0	(+)	(+)	0	0
Total biomass	148	77	175	341	192	165
Wastewater N load (kg N yr^{-1})	96	106	242	75	60	4
Total N load (kg N yr-1)	136	152	340	116	74	7

Table 2. Stable Isotope $\delta^{15}N$ and plant functional type.

Recharge Zone	**Species**	**Type**	**$\delta^{15}N$**
1	*Ceratophyllum demersum*	floating	1.27
3	*Ceratophyllum demersum*	floating	7.30
3	*Najas flexilis*	rooted	4.42
3	*Lemna minor*	floating	7.49
3	*Ceratophyllum demersum*	floating	6.41
3	Algal Epiphyte	floating	7.30
4	*Ceratophyllum demersum*	floating	5.57
4	*Najas flexilis*	rooted	4.27
4	*Lemna minor*	floating	5.91
5	*Ceratophyllum demersum*	floating	1.74
5	*Najas flexilis*	rooted	3.28
7	Algal epiphyte	floating	4.24
7	*Ceratophyllum demersum*	floating	0.07

Figure Legends

Fig. 1. Map of Oyster Pond. Inset shows area of recharge zones. Sampling sites (dots) are numbered by recharge area. Bathymetry is at 1 m intervals (3 m line bold). Shaded area shows estimated extent of beds of *Ceratophyllum demersum*.

Fig. 2. $\delta^{15}N$ values of floating (*Lemna minor*, *Ceratophyllum demersum*, algal epiphytes) and rooted (*Potamogeton* spp., *Najas flexilis*) plants versus wastewater N load (data from S. Good, this volume) received by the recharge areas.

Fig. 3. Mean biomass of *Ceratophyllum demersum*, other plant taxa, and all plant taxa versus wastewater N load.

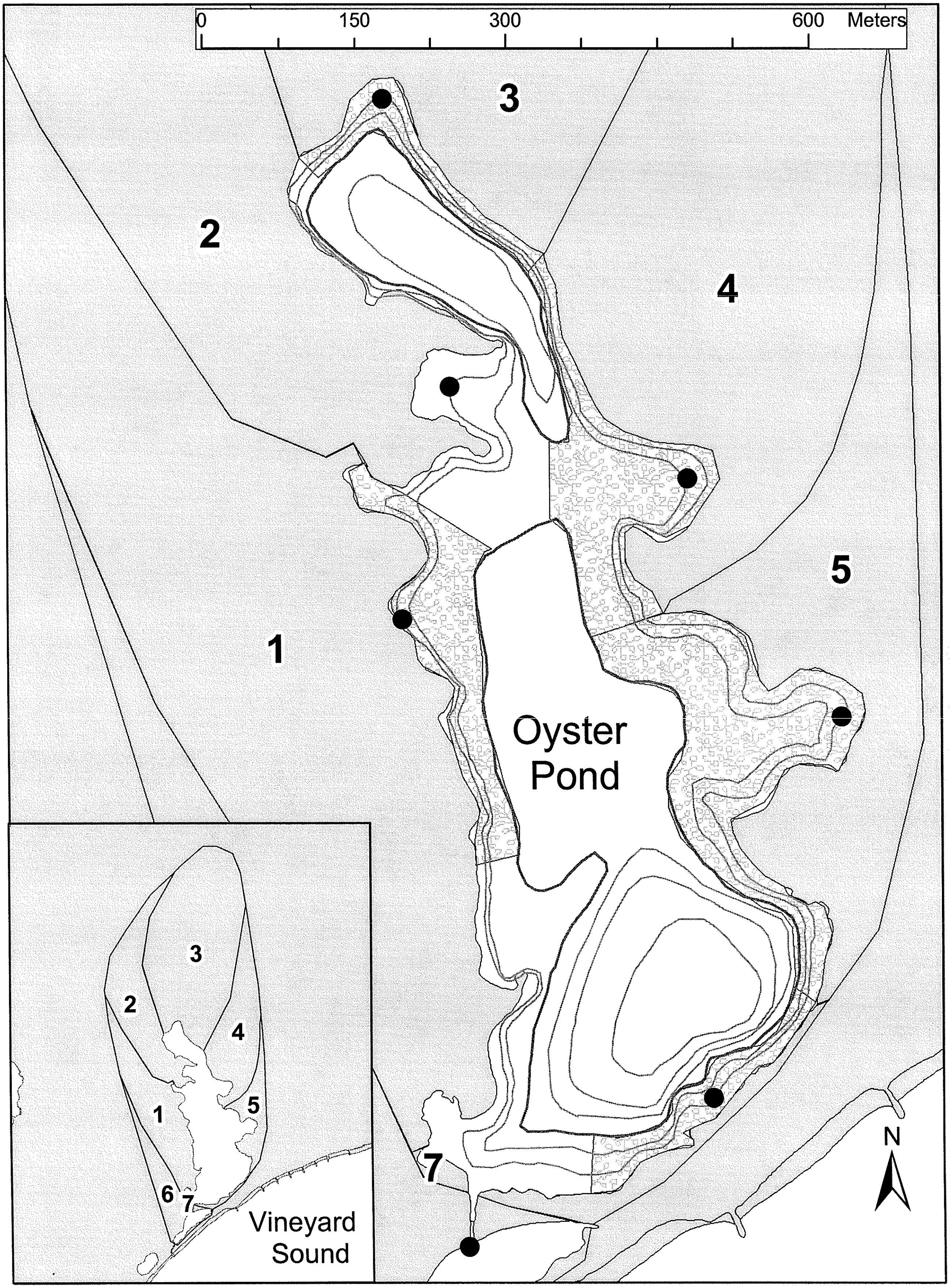
Fig. 4. Percent of SAV biomass contributed by different species versus wastewater N load.

Fig. 5. Abundances of Chironomids and Chaoborids versus wastewater N load.

Fig. 6. Abundance of Chironomids and Chaoborids versus plant biomass.

Fig. 7. Abundance of Ephemeroptera and Trichoptera as well as total # of taxa versus percentage of *Chara vulgaris* and *Ceratophyllum demersum*.

Fig. 1



0
150
300
600
Meters
3
2
4
5
1
Oyster
Pond
7
N
3
2
4
5
1
6
7
Vineyard
Sound

Fig. 2

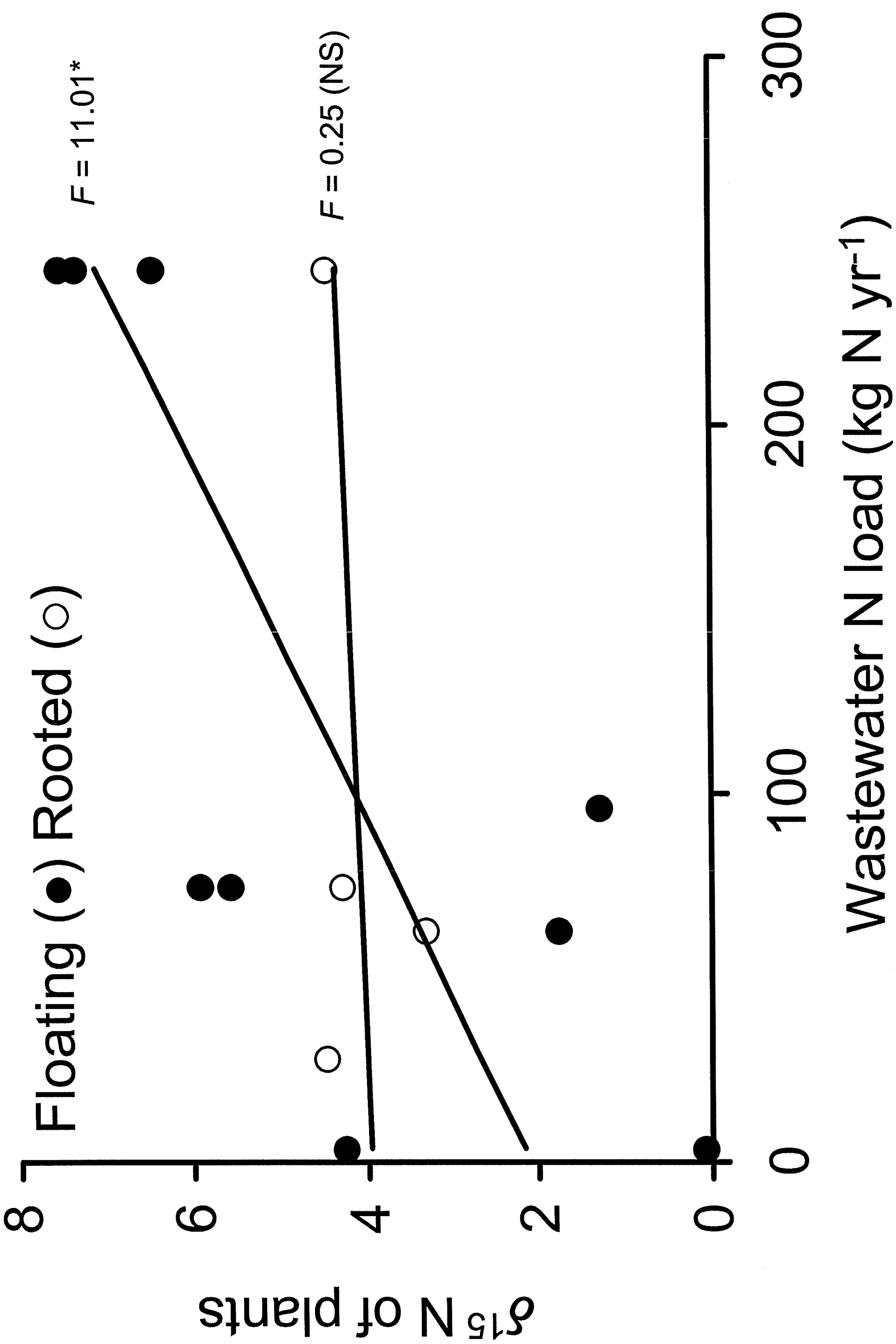

Floating (●) Rooted (○)
F = 11.01*
F = 0.25 (NS)
0
100
200
300
Wastewater N load (kg N yr-1)
8
6
4
2
0
δ15 N of plants

Fig. 3

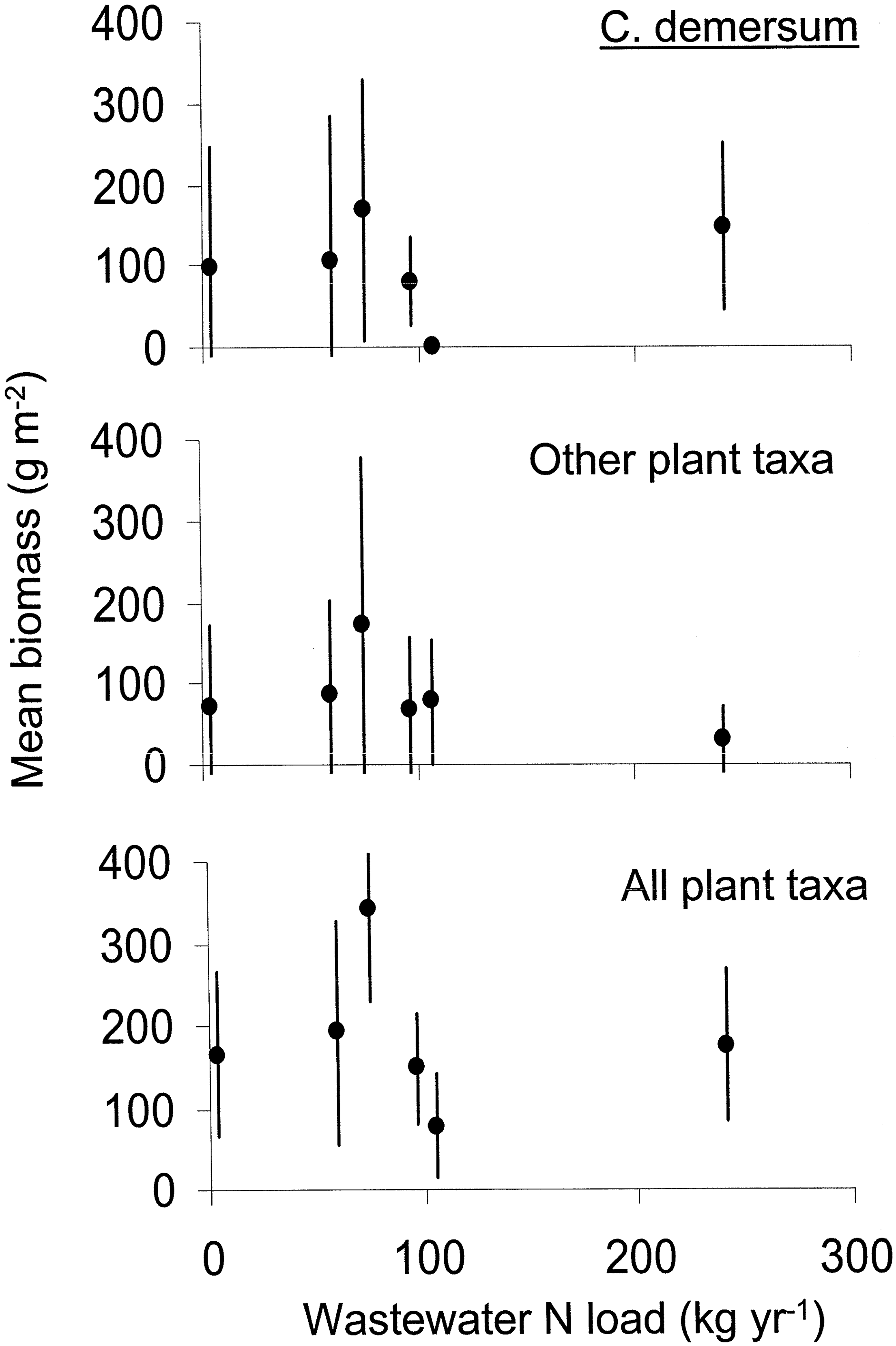
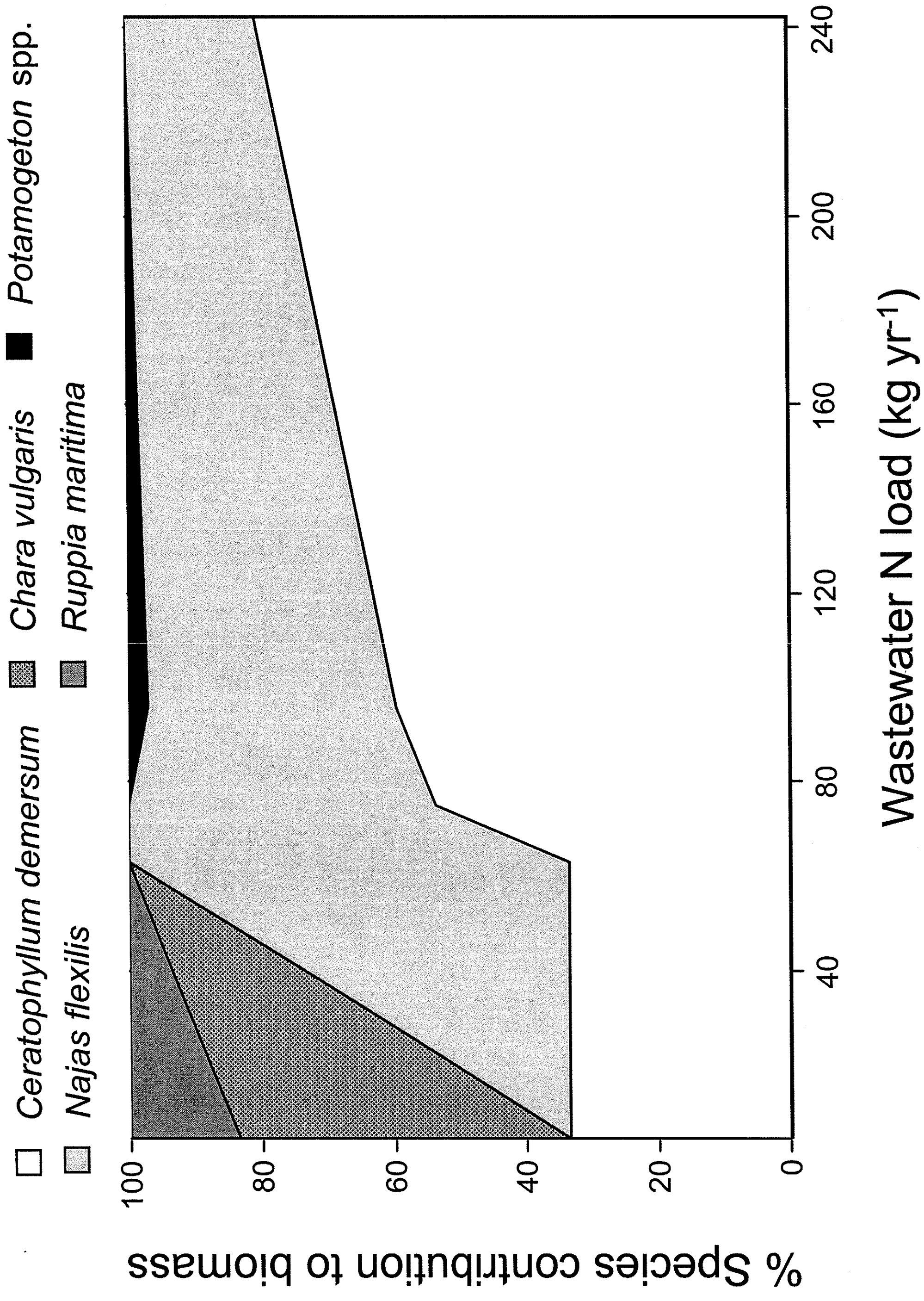
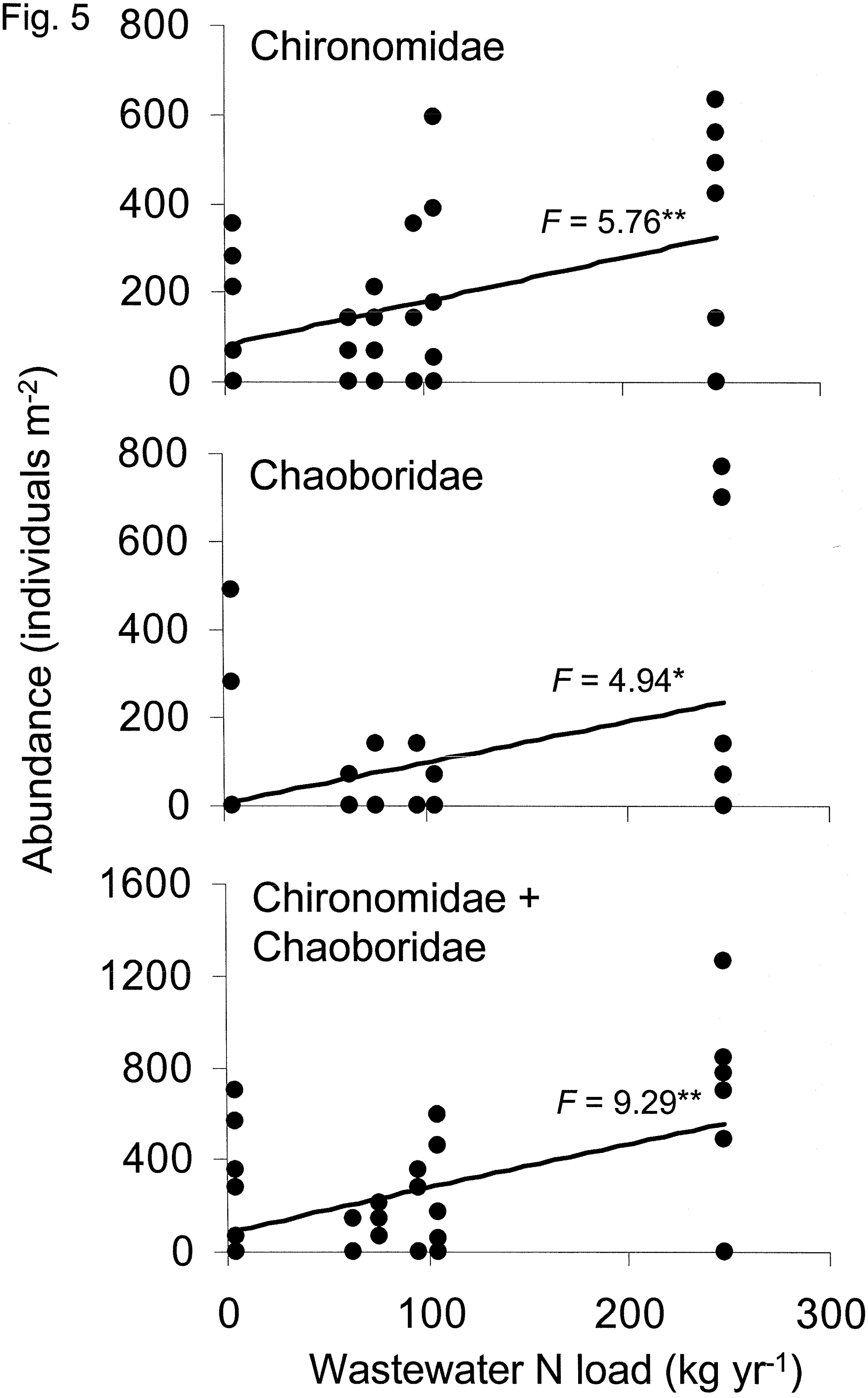

C. demersum
Other plant taxa
All plant taxa
Mean biomass (g m-2)
400
300
200
100
0
0
100
200
300
Wastewater N load (kg yr-1)

Fig. 4



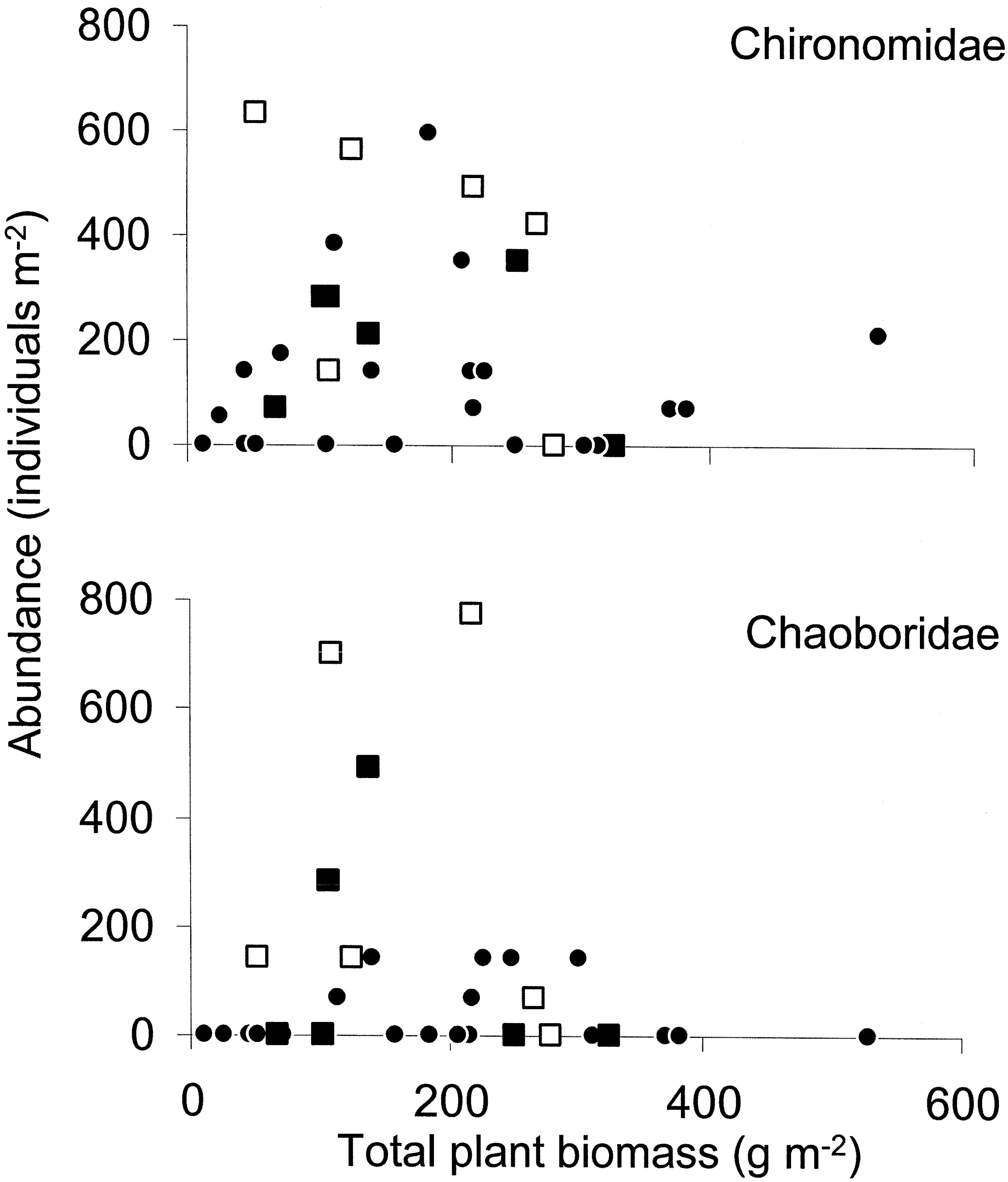
Ceratophyllum demersum
Najas flexilis
Chara vulgaris
Ruppia maritima
Potamogeton spp.
% Species contribution to biomass
0
20
40
60
80
100
40
80
120
160
200
240
Wastewater N load (kg yr-1)

Fig. 5



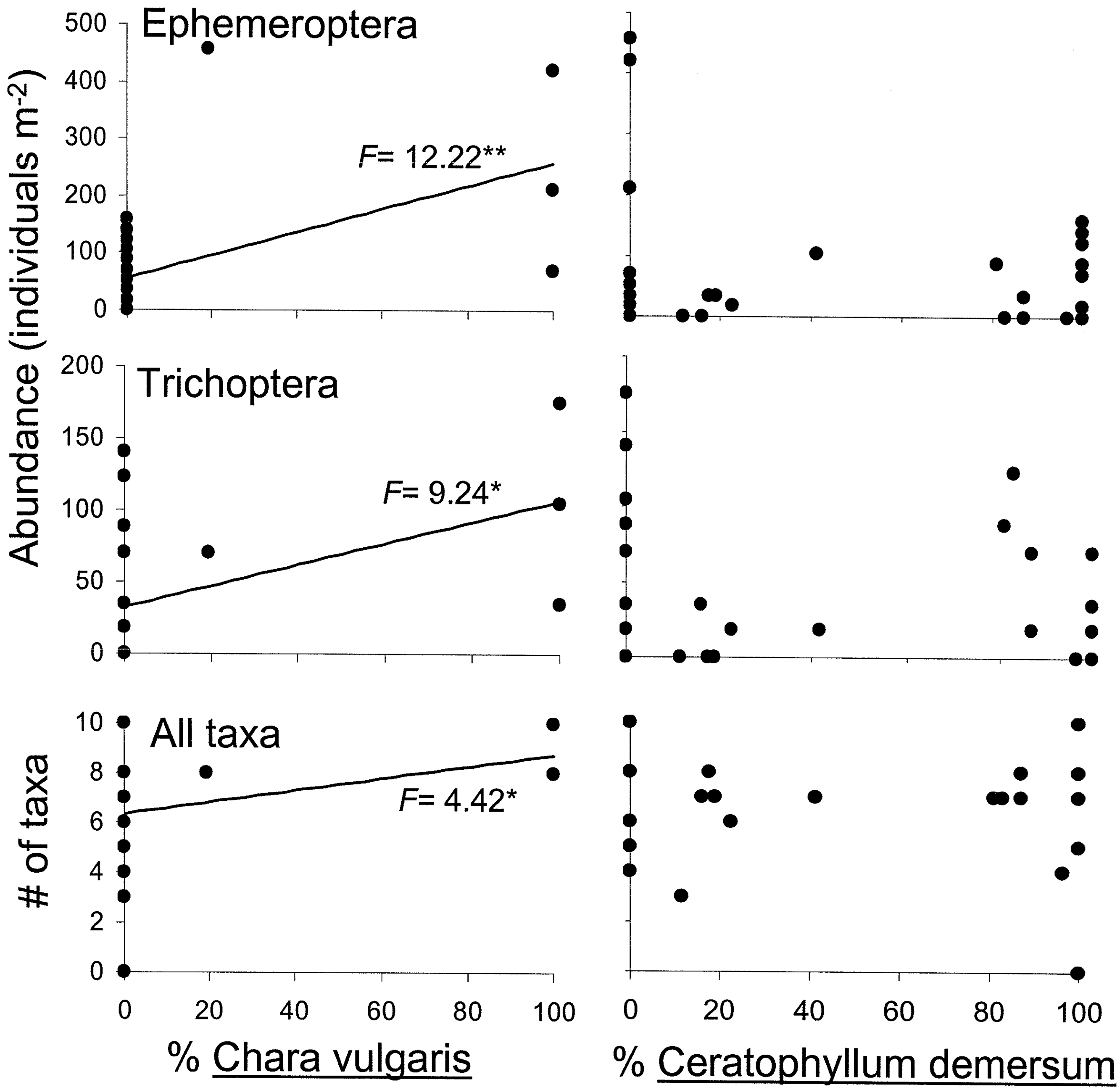
Chironomidae
F = 5.76**
Chaoboridae
F = 4.94*
Chironomidae + Chaoboridae
F = 9.29**
Abundance (individuals m-2)
800
600
400
200
0
1600
1200
0
100
200
300
Wastewater N load (kg yr-1)

Fig. 6



Wastewater N load: Maximum (□)
Minimum (■)
Other (•)

Fig. 7



Ephemeroptera
Trichoptera
All taxa
Abundance (individuals m-2)
# of taxa
F= 12.22**
F= 9.24*
F= 4.42*
500
400
300
200
100
0
150
50
10
8
6
4
2
20
40
60
80
% Chara vulgaris
% Ceratophyllum demersum