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The effects of biomanipulation on the biogeochemistry, carbon isotopic composition and pelagic food web relations of a shallow turf lake

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Abstract

The effects of fish removal on the biogeochemistry and lower-trophic level food web relations were studied in a shallow eutrophied turf lake. Biomanipulation led to an increase in transparency and macrophyte biomass and decrease in phytoplankton abundance, but zooplankton numbers did not increase. Moreover, fish removal resulted in high pH, high O_2, low CO_2 and more negative $\delta^{13}C_{DIC}$ values than expected, which is proposed to be the likely result of chemical enhanced diffusion with large negative fractionation (−13‰). By combining fluorescence activated cell sorting and isotope ratio mass spectrometry (IRMS) of fatty acids we were able to obtain group specific $\delta^{13}C$ signatures and to trace possible shifts in $\delta^{13}C$ resulting from fish removal. Fractionation values of green algae (20‰) and diatoms (22‰) were uniform and independent of treatment, while fractionation factors of filamentous cyanobacteria were variable between the treatments that differed in CO_2 availability. ^{13}C-labeling of the phytoplankton groups showed that biomanipulation led to increased growth rates of green algae and diatoms at the expense of cyanobacteria. Finally, the primary consumer *Chydorus* appeared to prefer cyanobacteria, whilst *Asplanchna* grazed predominantly upon eukaryotes.

1. Introduction

Most (turf) lakes in the Netherlands are highly eutrophic and the resulting algal blooms are an important reason for concern for lake management authorities. In general, phytoplankton biomass in these lakes is dominated by cyanobacteria. More or less permanent blooms of filamentous (*Planktothrix* or *Limnothrix*) cyanobacteria may be the result of their capacity to establish and maintain conditions that promote their own existence (Scheffer, 1998). Moreover, many cyanobacteria are fairly resistant to grazing by zooplankton, either through their relatively large (filamentous) size or through the production of toxins (Rohrlack et al., 1999; Gulati and deMott, 1997). The presence

of cyanobacterial blooms seriously interferes with lake use (e.g. for recreational purposes, drinking water supply), degrades ecosystem quality (Scheffer, 1998; Jeppesen et al., 1997; Moss et al., 1996) and is one of the main obstacles in lake restoration.

Initial efforts to restore eutrophied lakes focused on the reduction of nutrient loading, especially phosphorus. It was soon realized however that this by itself was insufficient to restore the lakes. Shallow lake ecosystems may exist in (at least) two alternative stable states: a clear state, dominated by submerged macrophytes and a turbid state, characterized by algal blooms. Both states resist change through feedback mechanisms between the turbid or clear state and biota in the lake, and these feedback mechanisms result in hysteresis in the recovery of lakes from eutrophication (Scheffer, 1998). Biomanipulation of lakes is a popular tool to assist lake restoration. It works by altering the food web structure of a lake and as such may remove obstacles for the recovery of lakes. In biomanipulation benthivorous and/or planktivorous fish (like bream) are removed from the lake, resulting in lower algal biomass by stimulation of zooplankton grazing pressure and reduced sediment resuspension. The expected result is an improved light climate and increased macrophyte settlement (Shapiro, 1972; Meijer and Hosper, 1997). However, former field experiments showed that, even when biomanipulation was combined with reduction of the external nutrient loading, the results of biomanipulation were variable and did not always lead to the desired improvement of water quality (Jeppesen et al., 1997). The reasons for failure of biomanipulation are not always understood, but may be explained by unstable relations in the food web as well as by the complexity of food webs in macrophyte-dominated lakes (Jeppesen, 1998). The perspective behind this study is that biomanipulation changes the lower food chain relationships, leading to changes in the carbon cycle, which are subsequently reflected in the C-isotopic values of dissolved inorganic and primary producers. Ultimately, the information on carbon isotope ratios (δ^{13}C) of the smaller size classes of the pelagic biota (phytoplankton and zooplankton), before and after fish removal may increase our understanding of ecosystem functioning and improve the predictability of the response of lakes to biomanipulation.

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In earlier studies, quantitative effects of biomanipulation on higher trophic levels have been described in detail (Meijer et al., 1999; Carpenter et al., 2001), but effects of fish reduction on CO_2, O_2, pH dynamics and carbon fluxes in the seston of especially shallow lakes are less well understood. Therefore in this study we investigated the effects of fish removal on the biogeochemistry of a Dutch turf pond and the interactions in the lower part of a food web, using state-of-the-art carbon isotope analysis combined with flowcytometry.

The rationale of using $\delta^{13}C$ in the analysis of lake biogeochemistry is that ^{12}C is preferentially taken up by phytoplankton, leaving a dissolved inorganic carbon (DIC) pool enriched in ^{13}C (more positive $\delta^{13}C$). Conversely, respiration of organic matter results in more negative $\delta^{13}C$ values. Consequently changes in the balance between production and respiration of organic matter are expected to be reflected in $\delta^{13}C_{DIC}$. Additionally, phytoplankton species may differ in isotopic composition, through variations in $\delta^{13}C_{DIC}$, growth rate (Laws et al., 1995), cell geometry (Popp et al., 1998), fixation pathways, nutrient availability (Bidigare et al., 1999) or other environmental parameters such as day length and CO_2 availability (Burkhardt et al., 1999). The isotopic signature of the zooplankton reflects that of their food source, with only a slight enrichment in the heavier isotope (^{13}C) at each trophic transfer (Rounick and Winterbourn, 1986; Goericke et al., 1994; Leggett et al., 1999). Therefore changes in the activity and composition of the phytoplankton, due to fish removal, are expected to be reflected in the isotopic signatures of zooplankton, the main consumers of green algae and cyanobacteria.

During the last decades stable isotopes have been used extensively to trace trophic interactions at higher levels of the food-webs (Peterson and Fry 1987; Kling et al., 1992; Keough et al., 1996), but methodological limitations have restricted species specific isotope analysis in the plankton community. New approaches based on the combination of biomarkers and stable isotope ratio mass spectrometry (Boschker and Middelburg, 2002) or the combination of flowcytometric cell-sorting and pyrolysis IRMS (Pel et al., 2003, 2004a) have recently been used to trace carbon flow through the microbial do-

main (Van den Meersche et al., 2004). Pel et al. (2003) demonstrated for the shallow Lake Loosdrecht that natural differences in isotopic signatures of 'algal' groups (green algae and cyanobacteria) can be used directly to study C transfer from primary to secondary consumers, and to assess preferential grazing by zooplankton. Schindler et al. (1997) observed that manipulation of predators and the consequent shift in grazers had a dramatic impact on lake CO_2 concentrations and the exchange of CO_2 across the air to water interface. They also observed that shifts in isotopic composition of algae (seston), zooplankton and fish were linked. Subsequently Carpenter et al. (2001) and Cole et al. (2000) confirmed the observations regarding the dynamic response of O_2 and CO_2 concentrations to biomanipulation. The aim of our study is to reveal potential shifts, caused by biomanipulation of a eutrophic, shallow (turf) lake, in (a) biogeochemical variables e.g. O_2 and CO_2 (b) δ^{13}C values of DIC, algae and zooplankton, and (c) trophic interactions between seston species. In contrast to other studies, which generally lump all seston for bulk analysis, we were able to identify the specific δ^{13}C per algal group by combining fluorescence-activated cell sorting (FACS) and Py-GC-IRMS (Pel et al., 2003, 2004a).

2. Material and methods

2.1. Study site

In October 2002 a biomanipulation experiment was started in the shallow lake Terra Nova (max. depth 1.5 m), which originates from turf digging in the 19th century (for characteristics see Table 1). The sediment is organic (detritus); the water column completely mixed and highly turbid for a large part of the year. In addition to nutrients already present, Terra Nova receives water by connection (at high water levels) to the nearby hypertrophic lakes Loenderveen and Loosdrecht (Gons et al., 1992). Water quality and biodiversity of Terra Nova have greatly deteriorated during the last 40 years, and the phytoplankton composition has shifted from a mixture of diatoms and

green algae to green algae and filamentous cyanobacteria. Nowadays, the cyanobacteria (*Limnothrix*; *Planktothrix*) make up more than 60% of the total phytoplankton community by number and the zooplankton is characterized by high population densities of detritivorous- and herbivorous species such as bosminids and small rotifers whilst large cladocerans like Daphnia are absent (G.ter Heerdt personal communication). Submerged macrophytes like *Nitella*, *Chara* and *Potamogeton* are missing, and floating macrophytes (*Nuphar* and *Nymphea spp.*) dominate the surface of the lake, in the open water as well as the littoral zone. Bream and roach are present in high densities (bream 89 and roach 9.7 kg ha^{-1}). In September 2002, two shallow turf ponds (average area=0.005 km^2; average depth=0.9 m) in Terra Nova were closed off from the rest of the lake by wooden dams, in order to prevent fish migration (Fig. 1). In January 2003, fish was removed from both ponds using nets and electrical fishing. Effectiveness of fish removal was determined by the mark-recapture method of Petersen (1896). Results were satisfactory with a total reduction of the planktivorous and benthivorous fish stock by almost 75% (source Witteveen and Bos). The southern turf pond was restocked with a known amount and composition of fish, reflecting the average Terra Nova densities (see earlier), whilst the northern turf pond was left without fish. For correct interpretation it is essential to realize that, besides reduction of the fish, also wind was reduced significantly in both enclosures. Reduction of the fetch may have reduced sediment resuspension and gas exchange across the air-water interface. Furthermore a reference location adjacent to the two closed turf ponds and in open contact with the main lake was monitored; this site was more wind exposed than the biomanipulated enclosures (but less wind exposed than the open water of Terra Nova), and fish conditions were left unchanged. From hereon we will refer to the different sample locations as – FW (– fish and – wind), – W (+ fish – wind) and R (reference; i.e. + fish + wind). Unfortunately a location without fish, but with wind conditions unchanged (– fish + wind) was not at hand.

2.2. Monitoring plankton and abiotic variables

Water samples were collected (bi) weekly, from February–December 2003 from the 3 locations, using a 1.5 m long tube sampler, which enabled us to sample the entire water column. From a mixed 30 L sample, a sub sample of 10 L was brought to the laboratory for analysis. Secchi depth, temperature, pH and oxygen concentrations were measured *in situ* (Multiline-P4 meter, WTW, Germany). In the laboratory, optical density (750 nm) was measured on a spectrophotometer (Halios-δ, Unicam, UK). To monitor phytoplankton abundance, Chl*a* fluorescence of green algae, diatoms and cyanobacteria was measured on a Phyto-PAM (Walz, Germany) and number of cells per algal group was determined on a Coulter Elite flowcytometer. For quantifying pelagic zooplankton, 4 L of field sample was filtered (33 μm; micro- and meso zooplankton), the residue was dissolved in 50 mL tap water, fixed with glutaraldehyde-formaldehyde (1% solution) and stored cold and dark. Later, zooplankton was counted microscopically (250×) using the sub sampling method of Kott (1953).

2.3. Stable isotope analysis

First, during 2003, concentrations and δ^{13}C values (^{13}C:^{12}C ratio) of DIC (C-source used by algae for primary production) were established every other week. Therefore 1.5 mL of raw field sample was transferred to 8 mL airtight bottles that had been flushed before with helium. To convert all HCO_3^- and CO_3^{2-} to CO_2 and to equilibrate CO_2 between the gas and water phase, the samples were acidified with 0.2 mL H_3PO_4 (2M). After 24 h, 0.4 mL of the gaseous phase was manually injected into a Euro Elemental Analyzer (Eurovector, Italy) coupled to a Finnigan Delta-S IRMS. A carbonate calibration series was run together with the samples (10, 20 and 40 ppm Na_2CO_3 in milli-Q, acidified and equilibrated similarly as the lake samples). Next, $CO_{2(aq)}$ and pCO_2 (ppmv) were derived from DIC and the in situ lake pH using pK values calculated according to Prieto and Millero (2002). Corrections for the discrimination of the heavy ^{13}C isotope (i.e. fractionation or ε), due to CO_2 solubility, temperature and gas/sample

volumes were made according to Baird et al. (2001), Miyajima et al. (1995) and Mook et al. (1974).

In addition $\delta^{13}C$ values of cellular fatty acids of the main phytoplankton (cyanobacteria, eukaryotes (green algae + diatoms)) and of zooplankton representatives were monitored at regular intervals, at the locations – FW, – W and R. Sample preparation by cell sorting, and isotope measurements of group-specific fatty acids was essentially conducted as described in Pel et al. (2004a). In these analyses we focused for diatoms on the group-specific $C_{20:5}$ fatty acid (Middelburg et al., 2000), whilst for green algae, cyanobacteria, *Microcystis* and flab (floating algal beds) the $C_{18:n}$ fatty acids were taken. At low algal densities, lake samples were concentrated by centrifugation (Jouan instruments, France) prior to flowcytometrical cell sorting (FACS). Pilot tests showed that cells remained intact and fluorescence was not significantly affected when centrifuged for 2×10 min, at 196 G. *Microcystis* and filamentous macro algae (flab) were not sorted on the flowcytometer, but colonies and filaments were handpicked under a microscope. Zooplankton used for isotope analysis were concentrated on a 33 μm filter and selected per species under a stereomicroscope (10× magnification). Depending on size and fatty acid richness, 1 to 20 individuals were used per sample. Isotope analysis of fatty acids was performed by in-situ pyrolytic methylation and IRMS linked gas chromatography. Instead of TMPAH (trimethyl phenyl ammonium hydroxide), used in previous studies as derivatisation reagent (Pel et al., 2003, 2004a), we used 0.5–0.7 μl TMSH (trimethyl sulfonium hydroxide; 0.25 M solution in methanol) in the present study, because of the much reduced isomerization side-effects of this reagent in the transesterification of poly-unsaturated fatty acids (Blokker et al., 2002). Carbon isotopic composition of the fatty acids is reported in δ-notation: $\delta^{13}C$ in parts per thousand (‰)=$[(^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{reference})-1] \times 10^3$ expressed relative to Vienna Pee Dee Belemnite. Reproducibility was <0.4‰ for FCM-sorted phytoplankton and ≤0.3‰ for handpicked zooplankton (Pel et al., 2003). Fatty acids were identified by their retention times using a known sample of *Limnothrix sp.* strain MR1 (Pel et al., 2004a). For an isotopic baseline of the pelagic food web in Terra Nova, we used the

main primary producers. To calculate the discrimination (factor ε) of the primary producers with respect to the in situ C-source, the average fatty acid-based $\delta^{13}C$ values of the algal groups were corrected for an offset of +9‰ observed between cellular fatty acids and total cell carbon biomass (see Pel et al., 2004a).

2.4. Growth rate measurements

In order to relate phytoplankton population dynamics to group-specific isotopic signatures, in-situ specific growth rates (μ_c(d^{-1}), where c is carbon) of cyanobacteria, green algae and diatoms for the 3 sites of Terra Nova were obtained monthly from 31 March till 25 November 2003. These growth rates were estimated from the rate of ^{13}C–CO_2 incorporation into the fatty acids over a 24h-period using the method of Pel et al. (2004b). To approach the in situ light conditions, the cells received the average light dose they received in the field (PAR), calculated according to Scheffer (1998), using Secchi depth, mixing depth and average light intensity of the previous 10 days. Light was provided by 4 to 8 Philips fluorescent light tubes (24 W/840). Samples for obtaining growth rate were taken at t_{0h} and t_{24h} after enrichment and isotope analysis of the DIC and phytoplankton was performed as described above (algae were sorted by FACS and their isotopic composition subsequently measured by Py-GC-IRMS). Growth rates were calculated according to Welschmeyer and Lorenzen (1984). Since sample preparation for isotope analysis is labor intensive and GC analysis time relatively long, samples were not analyzed in replicate.

2.5. Statistical analysis

Differences in the isotopic signatures of the main primary producer/plankton groups, within and between treatments, were analyzed for significance using a repeated measurement Anova ($p<0.05$) using Statistica (StatSoft, Inc., US, 2003). Prior to analysis, data were checked for normality and homogeneity of variance. Because isotope data of plankton groups did not always overlap between treatments and samples were mea-

sured only once, we primarily describe the observed trends between the treatments, qualitatively.

3. Results

3.1. General observations on the effects of biomanipulation

Biomanipulation and wind reduction had large effects on several biotic and abiotic parameters (Table 1). During the algal growing season, turbidities and phytoplankton densities (Chl*a* levels) were lowest in – FW, intermediate in – W and highest in R (Fig. 2a). In – FW, light penetrated to the sediment for most of the year, whilst in – W bottom visibility was never attained. In – W and R, the phytoplankton was represented by green algae (e.g. *Chlorococcales, Scenedesmus*) and diatoms (*Fragilara*), but was mainly dominated by high densities of filamentous or coccoid cyanobacteria (*Planktothrix, Aphanizomenon*, *Microcystis*). In – FW, low algal biomass was equally divided over cryptophytes, flagellates, larger diatoms (*Asterionella*) and green algal species (*Tetrastrum, Pediastrum*), whilst cyanobacteria played only a minor role. Finally, in all locations extremely high numbers of small unicellular green algae were present. Nutrient levels and temperature, did vary in time, but did not vary significantly between the treatments. Considering macrophyte presence; submerged macrophytes (*Elodea* and *Ceratophyllum*) reached highest coverage in – FW (~95% of the sediment surface area was covered by the end of August), and regularly seedlings of several nationally rare species (*Potamogeton obtusifolius, Chara globularis, Nitella flexilis*, *Najas marina*, *Stratiotes aloides*) were encountered. In – W, submerged *Elodea* and *Ceratophyllum* covered only one quarter of the turf pond in June, at which time floating macrophytes (*Nymphae* and *Nuphar*) took over for the rest of the season. In R, submerged plants were almost absent and the site was dominated by *Nymphae* and *Nuphar*. In all 3 locations, significant increases of flab, with species like *Melosira, Spyrogyra* and *Ulothrix*, occurred during spring to early summer (April–May 2003). Flab reached highest den-

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sities in - FW from 7 May till 16 June with 40% coverage. Accumulative numbers of zooplankton observed during 2003 were about twice as high in the presence of fish, i.e. higher in – W and R than in – FW (Table 2a). Also from monthly averages (Table 2b) it appears that, except in July 2003, zooplankton numbers were always lower in – FW. However, in – FW, large crustaceans (*Polyphemus, Semocephalus*, *and Pleuroxus*) were occasionally observed, but numbers remained low. In – W and R, reasonably high abundances of crustacean species (e.g. *Ceriodaphnia*, *D. cuculata, Chydorus*, *Bosmina*) were observed at regular intervals. Most striking, however, was the observation that small (herbivorous/detritivorous) rotifers such as *Keratella, Polyarthra, Anuraeopsis* and *Trichocerca* outnumbered all other species in all treatments. Cyclopoids copepods (adults/larvae) as well as *Asplanchna* and *Ceriodaphnia* occurred in comparable densities in the three treatments.

3.2. pH, O_2 and CO_2 dynamics

The effects of fish removal and the resulting extensive growth of macrophytes on $CO_{2(aq)}$ availability, O_2 saturation and pH in the water column were far-reaching (Figs. 2b and 2c). In – FW, pH increased from pH 7 to 8.5 between 15 April and 7 May. This increase was followed by a second, equally large increase from pH 8.5 to 10 within a two week period later in May. The alkaline state of the water lasted until 2 June, after which pH went down again to pH ≈7. In the same period substantial increases in O_2 saturation and decreases in pCO_2 occurred (Fig. 2b), which corresponded to observed increases in macrophyte biomass. It appears that in – FW, between 15 April and 2 June, production outbalanced respiration and the system was autotrophic (Fig. 2b). This is indicated by $[O_{2(aq)}]$ surpassing the 100% saturation level and $[CO_{2(aq)}]$ remaining below atmospheric levels (<370 ppmv). Decreased O_2 levels around the end of August may indicate increased mineralization of the flab and macrophyte biomass by bacteria and heterotrophic biota. In the treatments – W and R, pH never exceeded 8.6, and values averaged around ≈pH 7, throughout the year. For the largest part of the growing season, CO_2 production and O_2 consumption, appeared to be the general

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dominant mechanisms in these treatments, so net heterotrophy characterized these two systems. Nonetheless, O_2 and CO_2 levels did cross respectively the saturation and depletion levels for a short period between 25 June and 8 August 2003.

3.3. Stable isotope analysis of DIC, phytoplankton and zooplankton

In – FW we observed two exceptional large decreases in $\delta^{13}C_{(DIC)}$ (Fig. 2c); from ≈0‰ to −15‰ between 8 April–2 June and from −1‰ to −10‰ between 8 July–16 September. These excessive low $\delta^{13}C$ values occurred only in the absence of fish, while in the treatments – W and R, $\delta^{13}C_{(DIC)}$ values remained in the range from ≈2‰ to −4‰. Furthermore, average seasonal $\delta^{13}C$ values of DIC and CO_2 (mean −5.1‰) were more depleted in – FW than – W and R (mean −1‰). The decrease in $\delta^{13}C_{(DIC)}$ observed in – FW, was contrary to what we expected, namely enrichment in of the $^{13}C_{DIC}$ instead of depletion during autotrophic periods. The common reasoning for this is that under non-limiting CO_2 concentrations, the ^{12}C isotopes are always preferentially incorporated over the ^{13}C isotope during assimilation, thereby leaving a substrate (DIC) enriched in ^{13}C (Goericke et al., 1994).

Statistical analyses between the plankton groups, within one treatment, showed that in – FW, – W and R, diatoms, green algae and cyanobacteria did not significantly differ from each-other in isotopic signature (Anova, $p>0.05$). The $\delta^{13}C$ value of flab, however, significantly differed from that of the diatoms and green algae in – FW and of all other groups (incl. cyanobacteria and *Microcystis*) in – W. Also *Microcystis* $\delta^{13}C$ values deviated significantly from diatoms in – FW and other cyanobacteria in R.

Even though not all significant, the following trends in $\delta^{13}C$ values of green algae, diatoms and cyanobacteria were observed; in – FW and – W, $\delta^{13}C$ values of eukaryotic algae (−39‰ to −44‰) were more depleted than those of cyanobacteria (−33‰ to −36‰; including *Microcystis* −35‰), whilst flab was most enriched (−23‰ to −26‰). At the reference site, $\delta^{13}C$ values of eukaryotic algae and cyanobacteria fell in the same range (−38‰ to −41‰), whilst *Microcystis* was clearly more enriched (−33‰).

At all sites, diatoms were more depleted (−41‰ to −44‰) than green algae (−39‰ to −41‰). Cyanobacteria on the other hand were similarly depleted as eukaryotes in R but became more enriched in – W and – FW (see Appendix A). Table 3 shows that, after correction for the +9‰ offset between fatty acids and total cell carbon biomass, discrimination against the heavy ^{13}C isotope (fractionation; ε) became less in the sequence: diatoms >green algae> (filamentous) cyanobacteria >*Microcystis*> flab.

Figure 3b shows that in – FW and – W, with exclusion of the chydorids (−33.7‰), $\delta^{13}C$ values of zooplankton resembled the isotopic signature of green algae or diatoms rather than those of cyanobacteria, *Microcystis* or flab. At the reference site (R), the $\delta^{13}C$ of some zooplankton species either matched the isotopic composition of eukaryotes or the cyanobacteria, while in other species the diet appeared to be composed of several different but on average more enriched food sources (−36‰). Zooplankton isotope signature, however, did not differ significantly (ANOVA, $p>0.05$) within or between treatments.

3.4. Phytoplankton growth rates

Figure 4 shows that, irrespective of the treatment and time of year, specific growth rates of diatoms, green algae or cyanobacteria never exceeded $0.4\,d^{-1}$. Highest growth rates of green algae ($0.33\,d^{-1}$) and diatoms ($0.15\,d^{-1}$) in – FW and of cyanobacteria ($0.19\,d^{-1}$) in the reference were achieved on 25 June 2003. Mean μ_c values for the algal groups and treatments (see boxes in Fig. 4) indicate that green algae and diatoms were the dominant primary producers in the absence of fish whilst cyanobacteria became more important as wind and especially the impact of fish increased. In particular as turbidity increased in R, cyanobacteria appeared to grow faster (31 March). Seemingly, two growth peaks occurred in all treatments; – FW on 31 March and 25 June, and on 7 May and 25 June in – W and R. Unfortunately, datasets of Chl*a* and growth rate were not completely overlapping, since occasionally algal densities were too low to sort the clusters by FACS and growth rates were obtained on a monthly basis only. In general, with exception of the high growth rate of green algae on 31 March in –

FW, where subsequently phytoplankton biomass remained low, the majority of growth rate data seemed to coincide, with a – somewhat delayed – increase in phytoplankton biomass (Chl*a*).

4. Discussion

Before discussing in detail the isotope results, it is instructive to evaluate the monitoring results. In agreement with the majority of studies on biomanipulation in shallow, turbid lakes (Gulati and Van Donk, 2002; Van Donk et al., 1994; Moss et al., 1996), the removal of planktivorous/benthivorous fish in – FW led to increases in transparency, low Chl*a* concentrations and the return of submerged plants. Moreover, otherwise dominant filamentous cyanobacteria *Planktothrix* as well as *Limnothrix* (presumably strain *L. limnetica* MR 1 described by Rijkeboer et al., 1992) almost entirely disappeared, but cyanobacterial biomass was never fully replaced by other algae (green algae or diatoms). Large differences in cyanobacterial biomass between – W and R, may be the result of a larger wind fetch in R (Van Donk et al., 1994), leading to sediment resuspension and high turbidities, thereby promoting low light adapted cyanobacterial growth (Chorus and Bartram, 1999). We expected large-sized grazers to increase in – FW, in response to reduced predation pressure in the absence of fish (Hosper, 1993), which did not happen, however. We can only speculate on the causes, as for instance the sensitivity of *Daphnia* to elevated pH levels (Steiner, 2004). Absence of food (algae) is not likely to explain the conspicuous absence of larger grazers, given the fact that production rates in – FW were similar to the other sites (Fig. 4). Alternatively, the zooplankton population may never have recovered from the low abundances in spring or zooplankton feeding fish, still present after fish removal, may have consumed the plankton, as the summer proceeded. Instead of large cladocerans (Gulati and Van Donk 2002), we observed large numbers of small rotifers (e.g. *Keratella, Polyarthra*) in – FW, though still not as much as in the presence of fish. We can again only speculate about the possible reasons, such as the ability of these micro-heterotrophic organisms

to utilize alternative food sources, not/less accessible to the cladoceran population, e.g. detritus (Kling et al., 1992), but also the abundant small unicellular green algae, which possibly were effectively grazed upon by the high numbers of rotifers. In contradiction to the general opinion we cannot confirm that the predation of fish on invertebrates is a prime determinant of whether a lake will be clear and dominated by plants or turbid and dominated by phytoplankton (Hosper, 1993; Van Donk et al., 1994). In – FW, pelagic total phosphorous (TP) concentrations remained similar to those at the other locations, ranging from 0.03–0.1 mg L^{-1} (eutrophic-hypertrophic), but clear water was achieved and macrophytes settled. Evidently, nutrient reduction is just one of the key factors for (the initial) restoration to fail or to succeed. Numerous other authors (e.g. Scheffer, 1998; Jeppesen et al., 1997; Søndergaard et al., 2003) also state that at comparable nutrient levels, shallow lakes may be either clear or turbid depending on biological feedbacks in the system.

Shallow lakes have many pathways to channel C from primary producers through the food web and ^{13}C-analysis of sources and sinks has proved useful to decipher links between trophic levels within aquatic food webs (e.g. Kling et al., 1992).

What stood out in the isotope, analysis of the currently studied shallow lake, were the consequences of fish removal on the biogeochemistry of CO_2, O_2 and $\delta^{13}C_{(DIC)}$. While – W and R showed similar trends in these parameters, in – FW periods with increased primary production were accompanied by more extreme upward – and downward excursions of O_2 and pCO_2, respectively. Beside increased macrophyte and macro-algal growth, also low turnover rates of this biomass (low edibility compared to phytoplankton, however grazing by macrofauna like snails was not included in our study) may have caused pCO_2 levels to drop and O_2 and pH levels to rise in – FW. Although Terra Nova can be characterized as a net heterotrophic system during the largest part of the year at all 3 locations, the period between 22 April–25 June 2003 was characterized by O_2 increase and CO_2 decrease in – FW, and is recognized as a definite episode of autotrophic dominance. Hanson et al. (2004), Cole et al. (2000), Carpenter et al. (2001) and Schindler et al. (1997) reported a similar reduction in CO_2 upon biomanipulation.

Therefore, we may conclude that, also under the moderate to high TP conditions in Terra Nova, removal of fish temporarily changed the system in a net sink for atmospheric carbon, while under turbid, non-manipulated conditions the system was as a net source of carbon, consistent with observations by Cole et al. (2000).

5 Under high pCO_2, heterotrophic conditions, $\delta^{13}C_{DIC}$ usually becomes lighter, because respiration adds $\delta^{13}C$ depleted DIC (Goericke et al., 1994; Bade et al., 2004), while under low pCO_2 conditions, as in − FW, significant increases in $\delta^{13}C_{DIC}$ are expected. However, two periods with decreases in $\delta^{13}C_{DIC}$ were observed. The first lowering of the $\delta^{13}C_{DIC}$ value (April–June 2003) was most likely the result of chemically 10 enhanced diffusion of CO_2 at the air-water phase, which has earlier been described by Herczeg and Fairbanks (1987), and results in large fractionation. The high biomass of macroalgae − FW in this period, led to extreme low pCO_2 and high pH levels, thereby forcing invasion of CO_2 from the air into the water (see Ibelings and Maberly, 1998). Its subsequent binding with $OH^-_{(aq)}$ to $HCO_{3^-(aq)}$ probably led to a large negative frac- 15 tionation (approximately −13‰), opposite to the normal fractionation of about 8‰ in situations where $CO_{2(aq)}$ and $CO_{2(g)}$ are in equilibrium at atmospheric conditions. In Peter Lake, similar trends in $\delta^{13}C_{DIC}$, were observed by Bade et al. (2004) after fish reduction. The second drop in the isotopic values of DIC (July–September 2003), may next to modest chemical enhancement also partly have been caused by respiration of 20 the lake biota of organic detritus, rich in ^{12}C (Leggett et al., 1999; Rounick and Winterbourn, 1986; Rau, 1978), induced by increased temperatures. Or, alternatively, the oxidation of biogenic methane (range −40 to −60‰; Sierszen et al., 2004) from the sediment may have played a role in the depletion of the DIC. Because the ^{13}C depletion of the DIC is a seasonally dependent process, it complicates the use of $\delta^{13}C$ to 25 identify carbon sources in these systems (Bade et al., 2004; Herczeg and Fairbanks, 1987).

We were able to detect differences as well between the isotopic signatures of the primary producers and the different treatments (Fig. 4), which underlines the usefulness of this type of data to trace potential food preferences in the consumers. The variation

in $\delta^{13}C$ signature of the algae, may be a consequence of several factors including: the inorganic carbon species taken up (CO_2, HCO_3^-), concentration and $\delta^{13}C$ value of the C source, the specific growth rate (function of temperature, light, nutrients), cell geometry (cell permeability, cell size), C uptake kinetics (diffusion vs. active uptake) and the photosynthetic pathway (Laws et al., 1995; Peterson and Fry, 1987). In order to disentangle the contribution of these separate factors to the difference in isotopic composition of the algal groups, the fractionation factor ε (overall discrimination in the assimilation of carbon isotopes) was derived from the group-specific fatty acid ^{13}C signature (Table 3). Mean epsilons for diatoms (22‰) and green algae (20‰) were rather constant and independent of treatment and/or growth rate, suggesting that the algae in these groups were fixing CO_2, via the expected C_3 photosynthetic pathway and representing fractionation via Rubisco ($\varepsilon \approx 25-29$; Goericke et al., 1994). The epsilon for flab (macro algae), also based on the $C_{18:n}$ fatty acid, was ≈4‰. This observation of very low fractionation is consistent with studies describing "boundary-layer diffusion resistance" in sedentary or benthic organisms. Under low turbulent conditions unstirred layers, depleted in ^{12}C, form around the surface cells of sedentary organisms, forcing these cells to take up proportionally more ^{13}C than ^{12}C (LaZerte and Szalados, 1982; Goericke et al., 1994; France and Catanneo, 1998). On the contrary, mean ε for *Microcystis* (15-18‰) and other cyanobacteria (*Limnothrix* and *Planktothrix*; 11–20‰) showed more variability between treatments. These differences in isotope fractionation can be related to a number of factors and we discuss a few: One, differences in growth rates of the cyanobacteria in the different locations might have caused differences in ε. However, we would then expect fractionation to decrease as growth rate increased, while the results showed that growth rates (Fig. 4) and ε both increased from – FW to – W to R. Two, differences in CO_2 availability between – FW, – W and R, may have played a significant part in the variance in ε between the algal groups. While the eukaryotic algae fixed CO_2 via the C_3 pathway (Rubisco), cyanobacteria (*Microcystis*) might have additionally have utilized bicarbonate via carboxylation with decreased fractionation. This fixation pathway is used by phototrophic organisms adapted

to CO_2 limitations, such as cyanobacteria, under CO_2 limiting conditions (Goericke et al., 1994). And three, the relative importance of CO_2 uptake and active HCO_3^- uptake by cyanobacteria, as a consequence of the differences in CO_2 availability in the three different treatments (– FW, – W, R), may have determined the variance in ε of the cyanobacteria (see Table 3) (Cassar et al., 2004; Keller and Morel, 1999). If we would assume, for instance, ε to follow the relation: ε=21−fa×20, where fa is the fraction of HCO_3^- that is actively taken up and 21 relates to passive CO_2 uptake using Rubisco (like for diatoms and green algae), then according to the ε values in Table 3, cyanobacteria actively took up 50% in – FW, 25% in – W and nothing in R. Adopting another value for the CO_2 availability dependence (e.g. 10 rather 20) would change the actual estimate of active uptake, but not our conclusion that active uptake of bicarbonate by cyanobacteria under low pCO_2 might explain our results.

The sequence of algal fractionation factors observed in this study: diatoms > green algae >cyanobacteria> *Microcystis* > flab (Table 3), is consistent with other studies regarding the small fractionation by sedentary macro algae, but inconsistent with some recent reports, including our own, on micro algal isotope fractionation. Boschker et al. (2005) found that diatoms were more enriched than green algae in the upper part of the Scheldt estuary. Pel et al. (2003) found in Lake Loosdrecht, which is adjacent to Terra Nova, that cyanobacteria were more depleted than green algae and diatoms. Ultimately, there was enough resolution to distinguish the primary producers on basis of their fractionation value, but our limited understanding of factors governing isotope fractionation under natural conditions hampers the generalization of the effects of biomanipulation on isotopic signatures over multiple systems.

Biomanipulation had a clear effect on algal biomass, species composition and on the specific growth rates of the cyanobacteria, diatoms and green algae. In - FW, green algae grew faster than diatoms, which grew faster than cyanobacteria. Since this is not reflected in the biomass of the algal groups (Fig. 4), we assume specific loss factors like preferential grazing on green algae and small diatoms. Similar results were found for Lake Loosdrecht by Pel et al. (2003; see their Figs. 2 and 3). Despite the overwhelming

abundance of cyanobacteria, primary consumers depended heavily on the numerically rare eukaryotic algae that had higher growth rates than the cyanobacteria.

Considering the ^{13}C analysis of the zooplankton species, we briefly refer to some observations that allow us to attribute some primary consumers to certain food sources. In – FW the relative heavy $\delta^{13}C$ of *Chydorus* sp. implied that these species mainly grazed upon cyanobacteria, as did *Euchlanis* in Lake Loosdrecht (Pel et al., 2003), while in – W and R they switched to a mixture of eukaryotes and cyanobacteria. Alternatively, considering its semi-benthic habitat, *Chydorus* fed on an enriched food source near the bottom (benthic algae or epiphyton). The depleted $\delta^{13}C$ value of the large rotifer *Asplanchna*, in – FW, indicated a preference for the depleted green algae and diatoms, consistent with data on *Asplanchna* and *Brachionus* in Pel et al. (2003). In agreement with Pel et al. (2003), by looking at the stable isotopic values and the abundance of algae, rotifers and copepods, we hypothesize that the very abundant small unicellular green algae may have formed an important food source for the small rotifers, which were subsequently preyed upon by the cyclopoids. Although our study provides an unprecedented level of detail in the isotopic signatures in the primary producers, it appears that we are not able to determine the main food source. This can be due to the wide range in $\delta^{13}C$ of primary producers lowering the resolution or, as has been suggested before (Kerner et al., 2004), that multiple food substrates were consumed (omnivory) suggesting that the food web was not structured in a linear fashion and that generalist consumers prevailed.

5. Summary and conclusions

Biomanipulation in the shallow turf lake Terra Nova did follow the expectations with respect to increases of transparency, macrophyte settlement and decreases in phytoplankton abundance, but zooplankton numbers did not increase. Manipulation of fish resulted, at least during late spring/ early summer, in low pCO_2, high O_2 concentrations and anomalous negative $\delta^{13}C_{DIC}$ values due to chemical enhancement of CO_2diffusion

at high pH levels. This might be a more general phenomenon as it has been observed in biomanipulated Peter Lake as well (Bade et al., 2004). Removal of fish resulted in enhanced growth of green algae and diatoms at the expense of cyanobacteria. Green algae and diatoms mainly use Rubisco to fix CO_2 as reflected by their ε value, whilst cyanobacteria are also able to actively take up HCO_3^-, consistent with recent literature by Cassar et al. (2004) and Keller and Morel (1999). So, cyanobacterial fractionation depends on the treatment (-FW, -W or R), because CO_2 availability varied between treatments. *Chydorus* seemed to prefer cyanobacteria, whilst *Asplanchna* grazed upon eukaryotes. Finally, although exceptionally detailed isotopic values were obtained from phytoplankton and zooplankton in the field (see Appendix 1), by a unique combination of flowcytometrical cell sorting and isotope analysis, this was not sufficient to unravel the food web. Therefore we suggest the use of additional analytical tools, for instance additional isotopes (^{15}N; Veuger et al., 2005), fatty acid patterns, or the combination of deliberate tracer experiments with numerical models (Pace et al., 2004; Van den Meersche et al., 2004) to further unravel relations in the lower part of food webs.

Appendix A

Temporal variation in δ^{13}C values (‰) of the $C_{18:n}$ fatty acids value for green algae, cyanobacteria (*Microcystis*), flab and all assessed zooplankton species and the $C_{20:5}$ fatty acid for diatoms in – FW (Table 4), – W (Table 5), and R (Table 6) .

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Table 1. Several environmental variables for the three treatments; water chemistry variables are annual means based on regular measurements from February–December 2003. Ranges are the minimal and maximal values observed during this period.

	– FW			– W			R		
	Mean	Range	N	Mean	Range	N	Mean	Range	N
Secchi depth (cm^{-1})	85	65–100	19	65	35–90	19	65	30–90	19
CO_2 (ppmv)	2267	0.7–10152	19	1974	255–8379	19	951	72–2266	19
CO_2 (mM/L)	100.9	0.03–371	19	91.4	10.5–485	19	45.7	2.4–185.5	19
O_2 (% saturation)	89.0	40.8–158	17	83.8	48.5–133.8	17	95.4	58.5–146.1	17
P (mg P + PO_4^-/L)	0.10	0.05–0.2	16	0.15	0.06–0.3	17	0.14	0.07–0.2	17
N (mg N/L)	1.70	0.8–2.5	15	1.69	0.3–2.7	15	2.03	1.1–3.8	15
Chl*a* (mg/L)	26.0	1.7–48.2	18	40.0	6.5–87.4	18	57.8	5.5–119	18
pH	7.6	6.3–10.4	19	7.4	6.3–8.1	19	7.8	7.3–8.6	19
Temperature (°C)	15.2	2.9–24.5	19	15.4	2.8–25.2	19	15.8	3.7–25.9	19
Eukaryotes (nr.cells/mL)	26402	2092–103850	17	54770	9033–144834	18	48569	9909–116213	18
Cyanobacteria (nr.cells/mL)	16298	1922–88653	17	52500	9813–174135	18	86070	12303–287593	18

Table 2. Zooplankton densities at the 3 locations during 2003. **(a)** Sum of zooplankton individuals (n/L), per group and per key species. Cladocera include the species *Bosmina* and *Daphnia* and rotifers species from the genus *Keratella, Filinia, Polyarthra* and *Trichocerca*. Cyclopoid nauplli and adult cyclopoids are considered separately, because of their different diet; **(b)** total zooplankton numbers per month (n/L) (average of two counts per month).

	– FW	– W	R
(a)			
Total nr. yr^{-1}	12904	29992	31276
Cladocera	1965	6411	2688
Cyclopoida	3111	3832	2666
Nauplii	7533	10655	9105
Rotifera	14168	42401	50576
Bosmina	869	5176	1589
Ceriodaphnia	899	703	600
Chydorus	77	301	285
D.cuculata	32	197	182
Asplanchna	933	2930	1001
Polyphemus	86	6	16
(b)			
February	391	624	273
March	1178	1667	1449
April	1767	3765	3392
May	1313	2980	1844
June	1927	4773	4554
July	3181	2676	6342
August	2162	7585	4038
September	739	5207	6335
November	248	715	3050

Table 3. Mean fractionation (ε) for the specific fatty acids (FA) per algal group, per treatment, using $CO_{2(aq)}$ as C-source. For the green algae, cyanobacteria, *Microcystis* and flab/macroalgae groups the $C_{18:n}$ FA was used, whilst for diatoms the species specific $C_{20:5}$ FA was used.
Note: Epsilon values were corrected for the offset between fatty acids and total cell carbon content, by adding 9‰ to the $\delta^{13}C$ values (according to Pel et al., 2004a).

Location	Algal group	mean e	SE
– FW	Diatoms	22.4	1.4
	Green algae	19.7	1.1
	Cyanobacteria	11.4	2.8
	Flab	3.9	3.8
– W	Diatoms	23.4	1.4
	Green algae	21.7	1.0
	Cyanobacteria	17.8	1.4
	Microcystis	17.6	0.8
	Flab	4.5	0.7
R	Diatoms	22.6	3.1
	Green algae	19.9	1.9
	Cyanobacteria	20.8	1.1
	Microcystis	14.9	0.7

Table 4. Temporal variation in $\delta^{13}C$ values (‰) of the $C_{18:n}$ fatty acids value for green algae, cyanobacteria (*Microcystis*), flab and all assessed zooplankton species and the $C_{20:5}$ fatty acid for diatoms in – FW.

Date	Diatoms	Green algae	Cyanobacteria	Microcystis	Flab	Bosmina	Cyclopoids	Asplanchna	Daphnids	Chydorids	CO2 (aq)	DIC
12-Feb							-47.2	-48.3			-9.9	-1.3
3-Mar	-47.7	-43.1				-39.8	-38.4				-9.0	-0.3
24-Mar	-45.8	-42.7							-41.8		-8.1	0.1
22-Apr	-42.4	-40.2				-37.7		-38.0		-34.6	-13.4	-4.7
7-May	-48.7	-44.4									-14.9	-6.2
19-May		-33.3	-26.6				-33.8				-16.8	-9.0
2-Jun					-30.9		-39.5			-27.9	-22.6	-14.8
25-Jun	-48.8	-45.2					-38.7	-40.6		-38.5	-12.0	-6.7
8-Jul	-42.8	-44.6	-40.1				-36.3	-43.1	-42.9		-8.8	-0.9
5-Aug	-38.0	-39.2	-32.8		-24.2	-40.0	-40.9				-9.8	-2.6
1-Sep	-38.0	-42.3					-40.5			-34.0	-14.3	-6.6
29-Sep					-31.5		-31.6				-16.0	-8.2
25-Nov	-47.9	-42.8	-34.1		-22.7	-39.8	-37.9	-49.1			-13.4	-5.7
Mean	**-44.4**	**-41.8**	**-33.4**		**-27.3**	**-39.3**	**-38.5**	**-43.8**	**-42.3**	**-33.7**	**-13.0**	**-5.1**
SE	1.4	1.1	2.8		3.8	0.5	1.3	2.2	0.5	2.2	1.1	1.2

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Table 5. Temporal variation in $\delta^{13}C$ values (‰) of the $C_{18:n}$ fatty acids value for green algae, cyanobacteria (*Microcystis*), flab and all assessed zooplankton species and the $C_{20:5}$ fatty acid for diatoms in – W.

Date	Diatoms	Green algae	Cyanobacteria	Microcystis	Flab	Bosmina	Cyclopoids	Asplanchna	Daphnids	Chydorids	CO2 (aq)	DIC
12-Feb	-35.1	-42.3				-42.2	-46.9	-43.6			-10.1	-1.47
3-Mar	-47.7	-42.6				-39.5	-40.4	-35.8			-6.0	2.82
24-Mar	-39.5	-36.0				-33.1		-48.9	-38.5		-5.3	3.38
22-Apr	-44.1	-40.4				-34.2		-35.0		-35.6	-7.6	1.09
7-May	-40.7	-34.9		-35.4							-8.8	-0.65
19-May			-35.8				-38.2	-36.8			-11.3	-2.50
2-Jun					-21.5	-36.3	-36.1		-35.3	-36.7	-12.1	-3.38
25-Jun	-37.8	-40.5	-33.0	-34.2				-40.9	-39.2		-12.1	-4.07
8-Jul	-40.2	-39.3	-37.1	-35.3	-22.5		-39.3	-37.2		-37.9	-5.7	2.70
5-Aug		-36.9	-33.7	-33.5	-24.0		-40.9	-44.2		-39.6	-6.7	0.28
1-Sep	-42.6	-40.7	-35.9	-37.4			-39.4		-40.8	-39.8	-11.0	-3.06
29-Sep		-38.6	-32.9	-38.3			-42.5				-10.4	-2.75
25-Nov	-46.5	-46.4	-43.4			-43.6	-39.3	-47.8		-41.8	-11.7	-3.58
Mean	**-41.6**	**-39.9**	**-36.0**	**-35.7**	**-22.7**	**-38.1**	**-40.3**	**-41.1**	**-38.5**	**-38.6**	**-9.1**	**-0.9**
SE	1.4	1.0	1.4	0.8	0.7	1.8	1.0	1.8	1.1	0.9	0.7	0.7

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Table 6. Temporal variation in δ^{13}C values (‰) of the $C_{18:n}$ fatty acids value for green algae, cyanobacteria (*Microcystis*), flab and all assessed zooplankton species and the $C_{20:5}$ fatty acid for diatoms in R.

Date	Diatoms	Green algae	Cyanobacteria	Microcystis	Flab	Bosmina	Cyclopoids	Asplanchna	Daphnids	Chydorids	CO2 (aq)	DIC
12-Feb	-50.1	-49.0				-46.4					-7.1	1.45
24-Mar	-46.7	-42.1	-44.3			-39.7	-36.6	-42.3			-8.8	-0.10
22-Apr	-38.7	-36.7				-35.2	-34.4	-32.2	-31.1	-33.2	-8.7	0.10
19-May	-56.4	-48.5	-38.6				-43.5	-47.9	-40.9	-43.7	-11.4	-3.14
2-Jun				-33.5		-38.6	-36.9		-36.5	-32.0	-13.2	-4.51
25-Jun	-34.0	-36.0	-34.5					-40.6		-36.5	-12.6	-4.12
8-Jul	-33.3	-38.4	-42.5	-36.4		-39.5	-32.6	-38.1		-36.2	-8.5	-0.14
5-Aug	-32.0	-31.5	-35.8	-33.5			-31.1			-34.6	-8.1	0.71
1-Sep		-32.9	-38.7	-31.6						-36.5	-9.6	-0.81
29-Sep		-34.2	-39.0	-32.9		-38.0	-37.1	-36.7		-38.0	-9.0	-0.41
25-Nov	-37.6	-35.5	-41.0	-32.8		-40.5	-39.1	-41.8		-40.3	-8.0	0.38
Mean	**-41.1**	**-38.5**	**-39.3**	**-33.4**		**-39.7**	**-36.4**	**-39.9**	**-36.2**	**-36.8**	**-9.5**	**-1.0**
SE	3.1	1.9	1.1	0.7		1.3	1.4	1.9	2.8	1.2	0.6	0.6

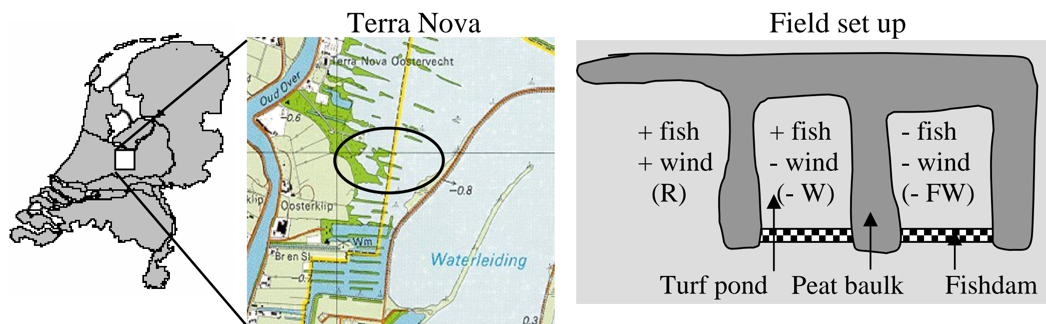

Fig. 1. Location of Terra Nova (the Loosdrecht Lakes, Utrecht, the Netherlands) and the design of the biomanipulation experiment in the field.

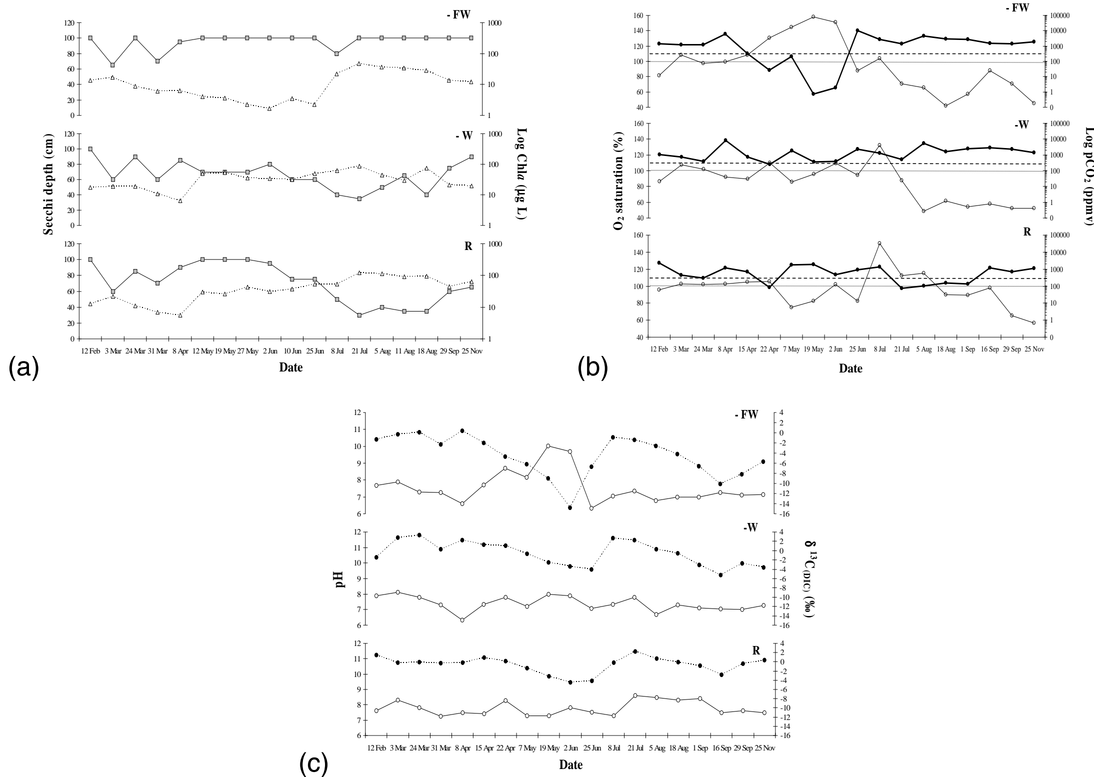

Fig. 2. **(a)** Transparency as Secchi depth (filled squares) vs. algal biomass as log total Chl*a* (open triangles) in the three treatments – FW, – W and R at Terra Nova from 12 February–8 December 2003. The sum of Chl*a* includes all algae groups present. **(b)** O_2 percentage (thin line with open symbols) and CO_2 in the water column (heavy line with solid symbols) at the 3 locations. Net autotrophy is indicated by levels higher than 100% O_2 saturation (thin horizontal line) and by CO_2 levels lower than ~370 μ atmosphere (heavy horizontal line **(c)** pH (open circles, solid line) and stable isotope (δ^{13}C) values of the primary C-source for algal growth; dissolved inorganic carbon (DIC) (solid circles, dotted line) for – FW, – W and R.

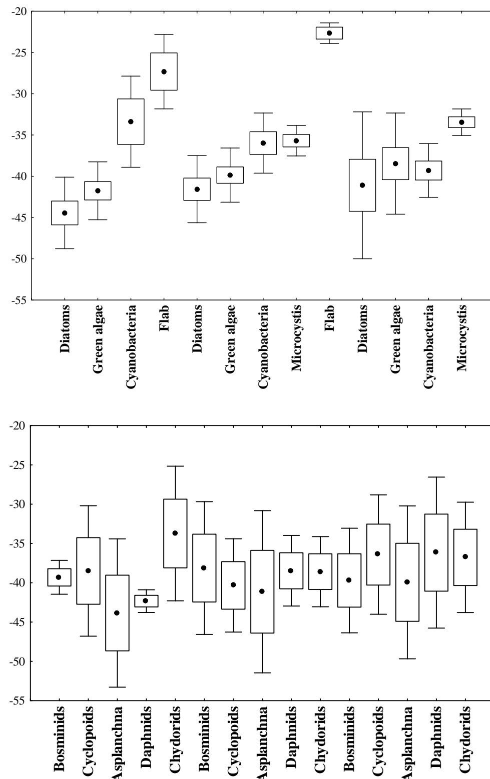

Fig. 3. Average δ^{13}C values (‰) of **(a)** the main primary producers and **(b)** the most abundant primary consumers in the three treatments – FW, – W, and R in 2003 in Terra Nova. Dots show mean δ^{13}C values per functional group, boxes give the 95% confidence intervals and whiskers the standard deviation. The δ^{13}C for all organisms is based on the $C_{18:n}$ fatty acid, but for diatoms ($C_{20:5}$).
Note: Filamentous cyanobacteria species such as *Planktothrix* and *Limnothrix* were separately analyzed from the cyanobacteria species *Microcystis*.

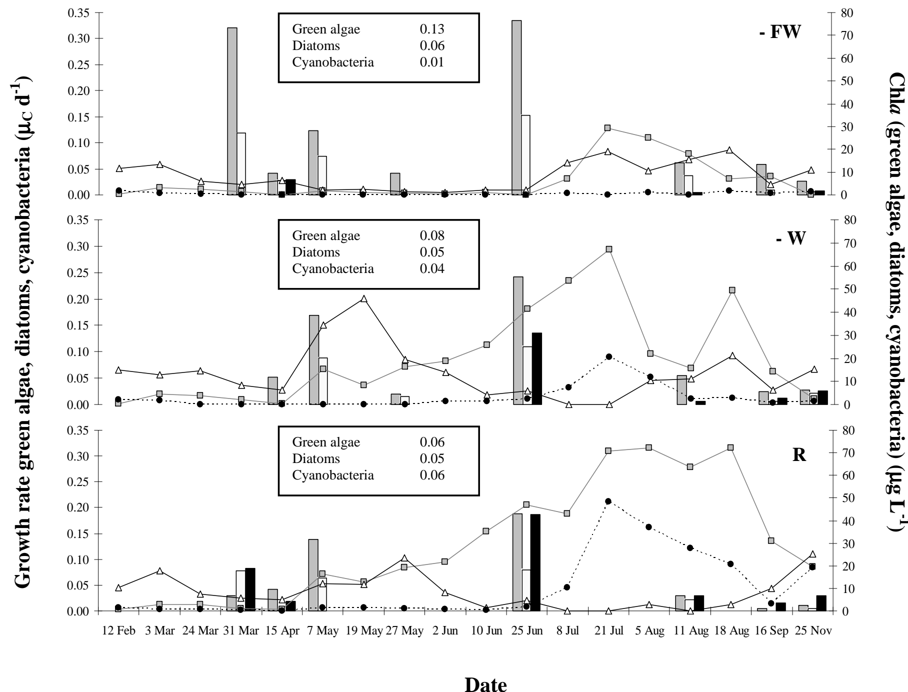

Fig. 4. Results of ^{13}C labelling of the different groups of phytoplankton, expressed as growth rates (μ_C (d^{-1})) and abundances of green algae, diatoms and cyanobacteria (Chl*a* in μg L^{-1}). Values represent average growth rates at ~18 and ~24 h after incubation; calculated as ^{13}C incorporation into the $C_{18:n}$ FA (Fatty Acid) of green algae (grey column) and cyanobacteria (black column), and the $C_{20:5}$ FA of diatoms (light grey column). Abundance of algae: green algae (grey line, squares), diatoms (black line, triangles) and cyanobacteria (dotted line, circles). Boxes represent the average seasonal growth rate (μ_C d^{-1}), per algal group, per treatment.