Published in final edited form as:

Limnol Oceanogr Methods. 2013 April 1; 11(4): 176–186. doi:10.4319/lom.2013.11.176.

Different mixing techniques in experimental mesocosms—does mixing affect plankton biomass and community composition?

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Abstract

Over the past four decades, mesocosm studies have been successfully used for a wide range of applications and have provided a lot of information on trophic interactions and biogeochemical cycling of aquatic ecosystem. However, the setup of such mesocosms (e.g., dimensions and duration of experiments) needs to be adapted to the relevant biological processes being investigated. Mixing of the water column is an important factor to be considered in mesocosm experiments because enclosing water in an artificial chamber always alters the mixing regime. Various approaches have been applied to generate mixing in experimental ecosystems, including pure mechanical mixing (e.g., using a disc), airlifts, bubbling with compressed air, and pumping. In this study, we tested different mixing techniques for outdoor mesocosms and their impact on plankton biomass and community composition. We compared mesocosms mixed with a disc, an airlift-system, and bubbling, and used a nonactively mixed mesocosm as a control. We investigated phytoplankton, ciliate, and zooplankton communities during a 19-d mesocosm experiment. Based on our results, we concluded that mechanical mixing with a disc was the most effective technique due to the undertow produced by lowering and lifting the disc. While no mixing technique affected seston biomass, zooplankton biomass was highest in the treatments mixed with the disc. The airlift treatments had the lowest relative share of small flagellates. However, no further differences in phytoplankton community composition occurred and no differences in zooplankton community composition existed between all actively mixed treatments.

Aquatic mesocosms (or enclosures) are often used in experimental aquatic ecology to conduct experiments at the ecosystem level and to investigate nutrient fluxes and ecological interactions (Lalli 1990; Oviatt 1994). Mesocosm studies provide the advantage of using natural communities in water bodies close to natural conditions, in which various environmental factors such as light (Demers et al. 1998; Diehl et al. 2002), nutrients (Tamminen; Goldman 1962; Harada et al. 1996; Schlüter 1998; Escaravage et al. 1999),

temperature (Berger et al. 2006; Sommer et al. 2007), and CO₂ (Riebesell et al. 2007) can be manipulated. Mesocosms are a powerful tool to link large field studies close to natural conditions with controlled small-scale laboratory experiments. They offer the possibility of investigating natural communities under close-to-natural conditions, maximizing the opportunities for control without losing the advantage of replication (Parsons 1982; Odum 1984; Riebesell et al. 2010). Over the past four decades, mesocosm studies have been successfully used for a wide range of applications and have provided a lot of information on trophic interactions and biogeochemical cycling in lakes (Flöder and Sommer 1999; Diehl et al. 2002; Reichwaldt and Stibor 2005) and marine systems (Oviatt 1994; Nejstgaard et al. 1997; Porter et al. 2004; Riebesell et al. 2008). Manipulative field- and laboratory-based experiments to test ecological theory increased during the last decades (Ives et al. 1996; Petersen et al. 2003) and searching for studies including ‘mesocosm’ or ‘enclosures’ from published sources using ISI Web of Science (conducted in March 2012) resulted in 1213 citations for ‘mesocosm’ and 1342 citations for ‘enclosures’ within aquatic categories (marine, freshwater biology, oceanography, fisheries, and limnology). This shows that mesocosm approaches are an often used tool in aquatic ecosystem research to investigate ecologically important questions and mechanisms behind such issues.

However, the setup of such mesocosms needs to be adapted to the relevant biological processes being investigated (Petersen et al. 1999, 2003; Petersen 2009; Riebesell et al. 2010). This means that the choice of mesocosm dimensions (depth, radius, and size), exchange rate, and the duration of the experiment needs to be adapted. A series of questions concerning these parameters have been the focus of long-term studies at the “Multiscale Experimental Ecosystem Research Center” (MEERC) at the University of Maryland (see Sanford 1997; Petersen et al. 1999, 2003; Petersen 2009). Furthermore, the mesocosms should be filled with the least disturbance possible (e.g., by using a certain type of pump) to reduce damage to (larger) organisms. The filling should also ensure a homogenous distribution of organisms, including the introduced communities, between experimental units. Mixing is another factor that needs to be considered in mesocosm experiments. Enclosing water in mesocosms always alters the mixing regime compared with the natural ecosystem (Riebesell et al. 2010). Thus, depending on the setup and hypothesis of the experiment, mixing in mesocosms can be of high importance (Sanford 1997). Mixing in outdoor mesocosm experiments can reduce sedimentation and avoid stratification of the water column. Mixing can also have a positive effect on primary production by maintaining cells in the photic zone, by releasing nutrients from benthic sources, and by decreasing the diffusion gradient around cells. On the other hand, mixing can negatively affect primary production by increasing turbidity from sediment resuspension. Various approaches have been applied to generate mixing in experimental ecosystems: using vertically oscillating plungers (Donaghay and Klos 1985), rotating paddles (Donaghay and Klos 1985), oscillating grids (Horwarth et al. 1993), airlifts (Reynolds et al. 1983; Svensen et al. 2001; Nejstgaard et al. 2006; Rijssel et al. 2007), bubbling with compressed air (Mohovic et al. 2006; Riebesell et al. 2007), or pumping (Demers et al. 1998; Mostajir et al. 1999; Whitehead et al. 2000; Fauchot et al. 2000; Fouilland et al. 2003; Mohovic et al. 2006; Roy et al. 2006; Riebesell et al. 2007). All these mixing techniques have advantages and disadvantages. However, there is no study comparing different mixing techniques directly,

and no one has examined the possible effects of these mixing techniques on plankton communities. Applications using air driven systems are generally considered to be gentler to organisms than pumping as pumps may damage larger organisms (e.g., zooplankton) due to high shear in the pump mechanism (Sanford 1997; Riebesell et al. 2010). Thus, we excluded pumps and tested three mixing techniques for their efficiency and their impacts on plankton communities: mixing with a vertically oscillating disc, with direct air-bubbling, and with an airlift system. As this study was designed to test the direct effect of mixing on plankton communities, we used relatively shallow mesocosms (2.5 m depth,) and did not focus on the intensity or frequency of mixing necessary to avoid stratification in deeper mesocosms. The mesocosms were incubated in the epilimnion of Lake Lunz. In this study, we address the following questions: (i) How efficient are different mixing techniques in homogenizing the water column? (ii) Are community biomass and/or composition affected by the mixing technique?

Materials and procedures

Experimental design

We tested the different mixing techniques and their impact on phytoplankton, ciliate, and zooplankton biomass and community composition in an outdoor mesocosm experiment. The experiment was carried out in Lake Lunz, a 33 m deep, low productivity lake in Austria. The mesocosms were cylindrical plastic bags (white PE foil, Renoplan), sealed at the bottom (conical end) and opened to the atmosphere, with a diameter of 0.95 m and a depth of 2.5 m, enclosing approximately 1800 L water, including the natural plankton community. We filled these 20 mesocosms by lowering and lifting and installed them on floating frames, which were connected to each other and fixed with anchors to the bottom of the lake. Mesocosms were filled within a few hours of each other to assure that initial plankton communities as well as nutrient conditions in the mesocosms were identical. To ensure phytoplankton growth in this oligotrophic system nutrients were added daily ($0.7 \mu\text{g P L}^{-1}$, $3.8 \mu\text{g N L}^{-1}$, and $5.7 \mu\text{g Si L}^{-1}$).

Four treatments (Fig. 1, with five replicates each) were established to test the effect of different mixing techniques on plankton communities: (a) nonactively mixing (as control), (b) mechanical mixing with a disc, (c) mixing by direct air bubbling, and (d) mixing by indirect air bubbling using an airlift system. The focus of this study was not to test the effect of energy applied to the system due to mixing but to test the direct, mechanical (possibly destructive) effect of the different mixing techniques on the plankton communities.

The mesocosms that were not actively mixed (a) served as control as no artificial effort was applied to mix these mesocosms. However, as we used mesocosms with flexible walls, energy from the lake (e.g., small waves) affected all mesocosms, including controls. Also, because the mesocosms were incubated in the well-mixed upper layer of the lake (thermocline was at approx. 9 m depth), all mesocosms were naturally gently mixed.

The disc for mechanical mixing (b) had a diameter of 0.755 m with eight holes of 0.128 m diameter each to allow the water to flow through during lowering and lifting in the mesocosms. For more detailed description of the disc, see Fig. 1 (e). For mechanical mixing,

the disc was lowered to the bottom and lifted to the surface (along the complete length of the mesocosm, Fig. 1b) five times per mesocosm per mixing event.

For mixing the ‘bubbling’ and ‘airlift treatments,’ compressed air (using a compressor with > 1 bar pressure, 120 Watt and 12 Volt; compressed air was distributed to all ‘bubbling’ and ‘airlift’ mesocosms at the same time and for the same duration) was released for 15 min to each mesocosm (with minimal pressure to assure gentle mixing). For direct bubbling, Fig. 1c compressed air was released from a tube (with a diameter of 0.013 m) in the center of the mesocosms approx. 0.1 m above the bottom (Fig. 1c). For indirect bubbling with the airlift-system, Fig. 1d compressed air was released from the same kind of tube as used for the ‘bubbling.’ The air was released into the lower part of a PE pipe with a diameter of 0.07 m and a length of 2.4 m (see Fig. 1d). This pipe was installed approx. 0.1 m above the bottom and fixed at the center of the mesocosms. The compressed air ascended in this pipe toward the surface of the mesocosms and moved the surrounding water in the same direction due to differences in density between water and air.

Previous tests (supported by the salt addition at the end of the experiment, see below) indicated that mixing in ‘bubbling’ and ‘airlift’ treatments was achieved after approximately 10 min. Mixing with the disc lasted 15 min for all mesocosms and was carried out at the same time as ‘bubbling’ and ‘airlift’ treatments were mixed. To assure equal start and end times for all mixing treatments, we decided for 15 min of ‘bubbling’ and ‘airlift’ mixing. Afterward, we started sampling. As the mesocosms were arranged randomly, the sampling was also conducted randomly.

All actively mixed mesocosms, treatments Fig. 1b-d, were mixed three times a day (8.00, 14.00, 20.00 h) to assure a homogenized water column. Temperature profiles (YSI 6920 v2) were measured on sampling days and light profiles (spherical quantum sensor LI-139SA; Licor) were measured three times during the experiment. The mean water temperature in the lake during the experiment (mean from surface to 2.5 m depth) was 16.1°C and ranged from 13.4°C to 20.8°C. The mean temperature ($\pm$ SD) for all mixed treatments at day 13 of the experiment before mixing was 14.1°C ($\pm$ 0.3°C) at the surface, 13.4°C ($\pm$ 0.3°C) at the bottom and 13.5°C ($\pm$ 0.1°C) after mixing at any depth. In the nonactively mixed treatments surface temperature was 15.2°C ($\pm$ 0.1°C) and temperature at the bottom was 12.0°C ($\pm$ 0.5°C), showing slight temperature differences between surface and bottom.

At the end of the experiment, salt solution was added to the mesocosm to test the efficiency of the mixing. To do this, 0.5 kg sodium chloride was predissolved in 2 L tap water and added to the water surface of each mesocosm, and the conductivity profile was measured for each mesocosm. In mesocosms mixed with the disc, homogeneous salt concentrations over the whole water column were measured after lowering and lifting the disc twice. In mesocosms mixed with direct air-bubbling and with the airlift-system, homogeneous salt concentrations were measured after 10 min. This showed that all mixing techniques were highly effective. However, in both treatments mixed by using compressed air (‘bubbling’ and ‘airlift’) the bottom 0.1 m layer was not completely homogeneous as the tubes for compressed air ended 0.1 m above the bottom of the mesocosms (see Fig. 1 (c) - (d)).

Light was comparable among all mesocosms: mean light intensities for all mesocosms at day 13 ranged from 900 (± 64 ; mean $\pm$ SD) $\mu\text{mol photons m}^{-2}$ at the surface to 250 (± 1 ; mean $\pm$ SD) $\mu\text{mol photons m}^{-2}$ at the bottom and thus no differences in light availability between the mesocosms due to possible sediment re-suspension by mixing occurred.

The experiment lasted for 19 days. However, as some of the mesocosms mixed with the disc exhibited holes above and below the water surface between day 16 and day 19 (the disc was uneven and abrasive at the outer edge) we decided to use the data measured at day 13 for further analysis. However, total phosphorus, seston POC, and zooplankton dry weight data are shown over the whole experimental period to give more detailed information about the development of these parameters in the course of the experiment.

Sampling and analyses

Sampling for nutrient analyses, phytoplankton, and ciliate parameters was performed at the beginning of the experiment (the day after filling) and every third day after that. In mixed treatments, samples were taken at 0.5 m depth immediately after mixing all mesocosms.

Phytoplankton samples were preserved with Lugol's solution, ciliate samples with Bouins solution, respectively. For all other analyses the water was immediately filtered through a 224 μm mesh screen. Total phosphorus (TP) was quantified by persulfate digestion followed by molybdate reaction (Wetzel and Likens 2003). Soluble reactive phosphorus (SRP) was also quantified by molybdate reaction. Samples for particulate organic carbon (POC) and particulate organic phosphorus (POP) analysis were filtered onto precombusted and acid-washed glass-fiber filters (Whatman GF/C). POC was measured by infrared spectrometry (C-Mat 5500, Ströhlein), and POP by molybdate reaction after sulfuric acid digestion (Wetzel and Likens 2003).

To determine cell numbers and biovolumes of phytoplankton, community samples were counted using an inverted microscope (Utermöhl 1958). Species richness was determined based on genus level. Species-specific cell volumes were calculated by approximation to simple geometrical bodies (Hillebrand et al. 1999). Community biovolumes were calculated as the product of single cell volumes with corresponding cell densities derived from Utermöhl counting. Diversity of phytoplankton communities based on biovolume data were calculated as the Shannon Index of diversity (H') according to Jost (2006). To test for effects of the different mixing techniques on the relative biovolume development of the phytoplankton communities, an algal response factor was calculated on a functional group level (according to Sarnelle 1992). This response factor is defined as the biovolume fraction of one functional group within the community at day 13, divided by the group's initial biovolume fraction at day 1. As green algal colonies and cyanobacteria represented less than 1% in all samples, these two functional groups were excluded from further analysis.

For ciliates, 100 mL whole water samples were fixed in 5% (final concentration) Bouin's solution and counted using an inverted microscope. Diversity of ciliate communities based on abundances was also calculated as the Shannon index of diversity (H'), species richness based on morphotypes. Zooplankton samples were taken every sixth day with a 55 μm net (approx. 95 L sample volume) and preserved with Lugol's solution. Species richness was

determined based on genus level. Zooplankton diversity was calculated as the Shannon Index of diversity (H') based on abundances (genus level). To test for effects of the different mixing techniques on the relative abundances within zooplankton communities, a zooplankton response factor was calculated on genus level for the most abundant ones (according to the calculation described in Sarnelle 1992). This response factor is defined as the relative abundance of one genus within the community at day 13, divided by the group's initial relative abundance at day 1. Zooplankton dry weight was determined after drying at 50°C to constant weight.

Statistical analyses were performed using R 2.14.1 (R Development Core Team 2011). Graphs were generated using SigmaPlot (11.0). If possible, parametric tests were done. If assumptions for parametric tests were not fulfilled, nonparametric tests were conducted.

Assessment

Nutrients and mixing

Initial nutrient concentrations were comparable between all mesocosms (e.g., total phosphorus [TP]: $4.2 \pm 0.1 \mu\text{g P L}^{-1}$; mean $\pm$ SE for all mesocosms). For more detailed information about initial conditions, see Tables 1 and 2. At day 13 of the experiment, TP concentrations did not differ among treatments (Kruskal-Wallis tests: $\chi^2 = 2.799$; $P = 0.424$, $df = 3$). However, over the course of the experiment (Fig. 2a, Table 3), the slopes of the linear regressions showed that TP concentrations were slightly higher in treatments mixed with the disc compared with all other treatments and that those were comparable with the theoretical expected concentration based on nutrient addition. Based on these results, we conclude that the mechanical mixing technique using the disc had the highest efficiency of mixing due to the undertow produced by lowering and lifting. The air-bubbling as well as the airlift system showed lower TP concentrations over time than the disc treatment, most probably because the tubes with compressed air were installed 0.1 m above the bottom of the mesocosms. Therefore, the last 0.1 m was not totally homogeneously mixed, and no compressed air reached the bottom. This was also observed when adding salt at the end of the experiment (*see* "Materials and procedures").

Plankton biomass and community composition

Initial seston particulate organic carbon (POC) concentrations (mg L^{-1}), used as a proxy for phytoplankton biomass, were the same in all mesocosms ($0.19 \pm 0.002 \text{ mg C L}^{-1}$). Seston POC concentrations increased during the experiment, reached maximum concentrations between days 13 and 16 of the experiment, and declined afterward. A maximum in seston biomass was already reached at day 13, and due to the possible damages in some mesocosm walls afterward (as described in "Materials and procedures"), all comparisons and statistical tests for differences between treatments were done for day 13. Seston POC concentrations did not differ between treatments at day 13 (Fig. 3a, Table 4). Phytoplankton Shannon Index (H' ; Fig. 3b) and species richness (Fig. 3c) also showed no differences among treatments at day 13. Focusing on the most abundant phytoplankton groups (flagellates, green algae, and diatoms; cyanobacteria contributed less than 1% of the biomass), no significant differences in the biovolume on day 13 existed among the treatments (Flagellates: $\chi^2 = 3.22$, $P = 0.4$;

Diatoms: $\chi^2 = 1.58$, $P = 0.7$; Green algae: $\chi^2 = 6.47$, $P = 0.09$). Additionally, we calculated phytoplankton response factors for all treatments, comparing the relative biomass development from day 1 until day 13 for different functional groups. For this response factor, we separated diatoms and flagellates into 'small' and 'large.' 'Small' were single cells with a diameter of less than 20 μm , and 'large' were either single cells with a diameter of more than 20 μm or colonies. Green algae were also divided into two categories, single cells and colonies including filamentous green algae. As colonies and filamentous green algae contributed to less than 1% of the total biomass this category was excluded for further analysis. Whereas large flagellates increased in relative biovolume during the experiment (mean = 0.27; 95% CI = 0.04), small flagellates decreased in relative biovolume (mean = -1.30; 95% CI = -0.19). The response factor of small diatoms was not different from zero (mean = 0.83; 95% CI = 1.10) whereas the relative biovolume of large diatoms (mean = -0.71; 95% CI = 0.54) and green algae (mean = -0.96; 95% CI = 0.33) decreased during the experiment. Treatment dependent differences existed only for small flagellates (Table 5); the response ratio of the airlift treatment was significantly lower compared with the response ratios of disc and bubbling treatments.

Ciliate abundances showed no significant differences among treatments at day 13 (Fig. 3d, Table 4). Ciliate Shannon Index (Fig. 3e) differed between the nonactively mixed and the airlift treatments (Table 4; pairwise comparisons using Wilcoxon rank sum test; $P = 0.048$). Ciliate species richness showed no differences among treatments (Fig. 3f, Table 4), although the ciliate Shannon Index tended to be lower in the nonactively mixed treatments compared to the actively-mixed ones (Fig. 3f). This might indicate a positive effect of mixing on ciliate diversity, or it might also indicate that ciliates were not evenly distributed in the nonactively mixed treatments and thus not adequately sampled in these mesocosms.

Zooplankton abundance increased during the experiment from 2.2 ± 0.2 individuals L^{-1} (mean $\pm$ SE for all mesocosms at day 1) to 7.0 ± 0.6 individuals L^{-1} (mean $\pm$ SE for all mesocosms) at day 13 and to 7.7 ± 0.7 individuals L^{-1} (mean $\pm$ SE for all mesocosms) at day 19. However, at day 13 of the experiment, zooplankton abundances did not differ among treatments (Fig. 3g, Table 4). Zooplankton Shannon Index also showed no treatment-dependent differences (Fig. 3h, Table 4), and species richness was the same in all mesocosms and therefore not displayed in Fig. 3.

Zooplankton dry weight increased with experimental duration (Fig. 2c), and mesocosms mixed with the disc had higher zooplankton dry weight on day 13 than all other treatments (Table 4, Fig. 3i). The most abundant groups at the beginning of the experiment were copepods (50.3%), followed by rotifers (*Asplanchna*, 36.9%), whereas cladocerans (*Bosmina*, 9.6%, and *Daphnia*, 3.4%, of the total abundance) were less abundant. During the experiment the relative abundances shifted completely and cladocerans became more abundant (day 13: 62.3% and day 19: 80.9%) while copepod and rotifer abundances decreased (copepods day 13: 20.2%, day 19: 12.9%; *Asplanchna* day 13: 17.7%, day 19: 6.3%).

The zooplankton response factor (Fig. 4b) illustrated this shift from copepod- and rotifer-dominated communities to cladoceran dominated communities during the experiment.

Additionally, this response factor shows the development of the relative abundances from the beginning of the experiment until day 13 for the different zooplankton groups: no treatment effects were observed for *Daphnia*, *Asplanchna*, or *Cyclops* whereas for *Bosmina* significant treatment effects occurred (Table 6). However, the following pairwise comparisons using Wilcoxon rank sum test (P adjustment methods Holm) showed no significant differences between the actively mixed treatments.

Discussion

It is well recognized that constraints of experimental mesocosms limits the extrapolation from artificial to natural systems (Oviatt 1994; Berg et al. 1999). Whereas fundamental scaling effects such as depth, volume, or radius of experimental units are well investigated (Sanford 1997; Petersen et al. 1999; Petersen 2009; Riebesell et al. 2010), different mixing techniques are still an often discussed topic when setting up mesocosm experiments. However, mixing is necessary to avoid stratification and/or reduce sinking losses in mesocosms experiments. As summarized in the introduction, different methods for mixing were used in different mesocosms studies until now and the concerns about disadvantages of certain methods are persistent. Petersen et al. (1998) summarized empirically determined effects of mixing on phytoplankton, microzooplankton (protozoa), and mesozooplankton (copepods). From negative to neutral to positive, all responses to mixing were found. As mixing levels differed between the individual experiments (from no mixing to levels atypical in nature), these data merely allow the conclusion that a variety of mixing effects on phytoplankton and zooplankton communities exists. Therefore, the aim of this study was to compare three mixing techniques and investigate the effects of these mechanical disturbances on phytoplankton, ciliate, and zooplankton communities. Based on existing literature, positive effects of mixing on large diatoms and negative effects of mixing on dinoflagellates were expected (Thomas and Gibson 1990; Svensen et al. 2001; Romero et al. 2012). However, our data showed negative response factors of large diatoms, especially in the actively mixed treatments. The response of flagellates in our experiment was size dependent. Small flagellates had a negative response while large flagellates had a positive response, and these responses were almost independent of mixing (with exception of the differences in the response factor of small flagellates between airlift treatments and disc or bubbling treatments; Fig. 4a).

According to Petersen (2009), major problems with air-bubbling are the injection of air into the water column resulting in higher dissolved gas concentrations and the potential damages by bubbles to 'delicate' organisms. Additionally, Petersen et al. (1998) showed that increasing the mixing intensity and time using a mixing impeller had a significant negative effect on copepod and gelatinous zooplankton abundance. Thus, we expected ciliate and zooplankton communities to be negatively affected by mixing, especially by mixing with compressed air. Ciliate species richness was lower in the nonactively mixed treatments compared to the actively mixed treatments. As mentioned before, this might be an effect of sampling: by sampling the nonactively mixed treatments also at 0.5 m, these samples might not be representative for the whole water column and actively moving ciliates might be underestimated in our samples. Zooplankton dry weight was also lower in the nonactively mixed treatments compared with the disc and the bubbling treatments. However, in this

case, differences in the vertical distribution of species were accounted for by sampling with net hauls from the bottom of the mesocosms up to the surface. As larger zooplankton can migrate, and may thus be patchy distributed on e.g., the very bottom of a mesocosm, especially during the day (when the mesocosms were sampled) this could result in artificially low estimates of abundances. It is also known that larger crustaceans are able to swim against currents, at least when they are not too strong (e.g., Titelman 2001 and several others). Thus, the reason for the higher number of zooplankton in the net may be partly explained by a possible effective displacement of larger zooplankton from the edges and the bottom of the mesocosms due to the relatively strong mixing for a much wider area by the disc, compared with the 'bubbling' or 'airlift' in a central point of the mesocosms. In fact, perhaps mixing with a wide disk before sampling mesocosms with a net may turn out to be much closer to the actual numbers of larger zooplankton species. Thus, differences in dry weight may actually be a partially artefact and thus not be a strong argument for actual difference between the mesocosms. Instead that would further support the conclusion that all of the methods used here do not seem to influence the outcome of the experiments in any significantly different way.

Our data suggests that mixing had no significant effect on seston biomass and no or positive effect on zooplankton dry weight (Fig. 3i). In the phytoplankton communities, the relative biomass of large diatoms, green algae, and small flagellates decreased whereas the relative biomass of large flagellates increased. However, no clear effect of a certain mixing technique was observed on the phytoplankton community composition (Fig. 4a). Zooplankton dry weight was highest in the disc and the bubbling treatments, and we observed a shift from copepods to cladocerans. Over the course of the experiment, no statistically significant difference between the actively mixed treatments in zooplankton community composition was seen (Fig. 4b).

The efficiency of mixing was high for all tested mixing techniques. However, using compressed air for mixing (both bubbling and airlift) resulted in a 0.1 m depth layer at the bottom of the mesocosm that could not be mixed. On the other hand, homogenizing the water column using a disc means that this has to be done in regular intervals. Installing very deep mesocosms for an experiment or facing stratification due to temperature or salinity might also necessitate more frequent mixing than in our experiment. This means that using a disc for such applications requires an installation that assures frequent automatized mixing with a disc in regular intervals. Increasing the intervals of mixing by using compressed air is possible but can affect the plankton communities more than observed in our study (Thomas and Gibson 1990; Petersen et al. 1998; Romero et al. 2012). Further studies about the intervals and intensities necessary for mixing deep and stratified mesocosms with compressed air are necessary to obtain more information about possible impacts of these mixing approaches on plankton communities.

Comments and recommendations

Based on our results, we conclude that we observed differences between seston biomass of nonactively mixed and any actively mixed treatment. However, depending on the mixing technique, we observed differences in the phytoplankton response factor, (the relative

development of biomass), of some phytoplankton groups. Ciliate abundances, species richness, and Shannon Index showed no differences between different mixing techniques. Zooplankton dry weight was highest in treatments mixed with the disc, whereas zooplankton community composition showed no differences between non-actively mixed treatments or any actively mixed treatments.

Mixing the mesocosms with a disc turned out to be the most effective method to homogenize the water column due to the undertow produced by lowering and lifting.

Acknowledgments

This study was funded by the Austrian Science Fund (FWF P22978-B17). We thank Christian Preiler, Andreas Ganglbauer, and Achim Weigert for technical support, Keeley MacNeill and three anonymous reviewers for very helpful comments on this manuscript.

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Fig. 1.

Scheme of experimental setup: (a) Non-actively mixing: no active mixing was conducted in these mesocosms. The depth of all mesocosms in this experiment was 2.5 m, and the diameter was 0.95 m. (b) Mechanical mixing with a disc: the disc was lowered to the bottom of the mesocosms and lifted five times for each mixing. (c) Mixing with direct air bubbles: compressed air was released (for 15 min) from a tube (0.013 m diameter) in the center of the mesocosms approx. 0.1 m above the bottom. (d) Airlift system where compressed air was released (for 15 min) from a tube (0.013 m diameter) in a standpipe (2.4 m length and 0.07 m diameter) made of polyethylene in the center of the mesocosms approx. 0.1 m above the bottom. All actively mixed mesocosms (b)-(d) were mixed three times a day (8.00, 14.00, 20.00 h) to assure a mixed water column. (e) Specifications of the disc used for mixing: the diameter of the disc was 0.755 m, thickness 0.02 m, and diameter of the holes 0.128 m. The diameter of the inner circle with holes was 0.25 m and the diameter of the outer circle with holes was 0.51 m.

Fig. 2.

Total phosphorus (TP) concentrations ($\mu\text{g P L}^{-1}$): (a) seston particulate organic carbon (POC) concentrations (mg C L^{-1}), (b) and zooplankton dry weight (mg L^{-1}) in the different treatments during the experiment were given. The solid black lines in (a) show the theoretically expected TP concentrations based on the initial concentrations (day 1) and a daily nutrient addition ($0.7 \mu\text{g P L}^{-1}$). For linear regression parameters of TP concentration over time, see Table 3.

Fig. 3.

Phytoplankton (a)-(c), ciliate (d)-(f), and zooplankton (g)-(i) parameters as means ($\pm$ SD) for all treatments (nonactively mixing, disc, bubbling, and airlift) at day 13 of the experiment. (a) Seston POC concentrations (mg L⁻¹) as proxy for phytoplankton biomass, (b) phytoplankton Shannon Index (H'), (c) phytoplankton species richness, (d) ciliate abundance (individuals L⁻¹), (e) ciliate Shannon Index (H'), (f) ciliate species richness, (g) zooplankton abundance (individuals L⁻¹), (h) zooplankton Shannon Index (H'), and (i) zooplankton dry weight (mg L⁻¹). Statistically significant differences were detected only for ciliate Shannon Index (H') (e); here the nonactively mixed and the airlift treatments differed (pairwise comparisons using Wilcox rank sum test; $P = 0.048$). All statistical parameters are displayed in Table 4.

Fig. 4.

Phytoplankton response factor (a) and zooplankton response factor (b) for the most relevant groups in terms of community composition for all treatments. (a) The phytoplankton response factor was calculated to compare the relative biomass development from day 1 until day 13 for the different treatments. Diatoms or flagellates were defined as 'small,' if they occurred as single cells with a diameter of less than 20 μm , and as 'large,' if they occurred either as single cells with a diameter of more than 20 μm or as colonies. Green algae were also divided into two categories: single cells and colonies (including filamentous green algae). As colonies and filamentous green algae contributed less than 1% to the total biomass, this category was excluded for further analysis, as well as cyanobacteria, which also contributed less than 1% to the total biomass. (b) The zooplankton response factor was calculated to compare the development of the relative abundances for *Daphnia*, *Bosmina*, *Asplanchna*, and for *Cyclops* for the different treatments from day 1 until day 13. All statistical parameters are displayed in Table 5.

Table 1

Summary of initial parameters pooled over all meso-cosms after filling the mesocosms at the beginning of the experiment are displayed as means with $\pm$ SE (day 1).

Parameter	Initial concentrations
NH ₄	11.2 (0.7) $\mu\text{g L}^{-1}$
NO ₂	3.0 (0.2) $\mu\text{g L}^{-1}$
NO ₃	1062.6 (2.5) $\mu\text{g L}^{-1}$
SRP	2.2 (0.1) $\mu\text{g L}^{-1}$
TP	4.2 (0.1) $\mu\text{g L}^{-1}$
Seston POP	0.69 (0.06) $\mu\text{g L}^{-1}$
Seston Chlorophyll <i>a</i>	0.47 (0.01) $\mu\text{g L}^{-1}$
Seston POC	0.19 (0.002) mg L^{-1}
Seston molar C:P	713.2 (64.9)
Phytoplankton biovolume	4.79×10^7 (8.7×10^6) $\mu\text{m}^3 \text{L}^{-1}$
Zooplankton dry weight	1.4 (0.1) mg L^{-1}

Table 2

Initial parameters after filling mesocosms at the beginning of the experiment. Means calculated over all mesocosms are displayed with $\pm$ SE (day 1).

Depth	Temperature (°C)	O ₂ (mg L ⁻¹)	pH	Conductivity (μS cm ⁻¹)
0 m	20.7 (0.1)	9.8 (0.2)	8.55 (0.1)	238.4 (0.6)
1 m	20.5 (0.1)	9.9 (0.3)	8.53 (0.04)	238.2 (0.5)
2 m	19.6 (0.3)	10.6 (0.2)	8.60 (0.04)	237.2 (0.5)

Table 3

Linear regression parameters for total phosphorus concentrations in the different treatments over the course of the experiment ($\text{TP } \mu\text{g L}^{-1} = \text{intercept} + \text{slope} \cdot \text{d}$), as shown in Fig. 2(a). The theoretically expected TP concentrations (based on initial concentrations and the amount of daily nutrient addition) were $\text{TP } (\mu\text{g L}^{-1}) = 3.86 + 0.7 \cdot \text{d}$.

Treatment	Intercept ($\pm$ SE)	Slope ($\pm$ SE)	r^2	P
Non-active mixing	5.30 (0.88)	0.47 (0.08)	0.54	<0.001
Disc	3.83 (0.50)	0.68 (0.04)	0.89	<0.001
Bubbling	4.66 (0.60)	0.43 (0.05)	0.68	<0.001
Airlift	5.34 (0.66)	0.40 (0.06)	0.45	<0.001

Table 4

Results of Kruskal-Wallis test for different plankton parameters (displayed in Fig. 3). Pairwise comparisons, using Wilcoxon rank sum test (P adjustment methods Holm), for ciliate Shannon Index showed differences between the treatments 'non-active mixing' and 'airlift' ($P = 0.048$). For zooplankton dry weight, differences existed between the treatment 'disc' and all other treatments ($P = 0.048$), and between 'bubbling' versus 'airlift system' ($P = 0.048$).

Parameter	χ^2	df	P
Seston POC (mg L ⁻¹)	4.394	3	0.222
Phytoplankton Shannon index (H')	3.480	3	0.323
Phytoplankton species richness	3.071	3	0.381
Ciliate abundance (ind. L ⁻¹)	3.109	3	0.375
Ciliate Shannon index (H')	8.234	3	0.041*
Ciliate species richness	6.767	3	0.080
Zooplankton abundance (ind. L ⁻¹)	4.406	3	0.221
Zooplankton Shannon index (H')	3.754	3	0.289
Zooplankton dry weight (mg L ⁻¹)	14.200	3	0.003*

* Statistically significant results.

Table 5

Parameters of ANOVA for phytoplankton response factor based on defined functional groups [Fig. 4(a)].

Phytoplankton functional group	F	df	P
Small diatoms	0.582	3	0.640
Large diatoms	1.239	3	0.342
Green algae	2.678	3	0.082
Small flagellates	4.388	3	0.020*
Large flagellates	0.569	3	0.644

* Statistically significant results.

Table 6

Parameters of Kruskal-Wallis test for zooplankton response factor based on genus level [Fig. 4(b)].

Zooplankton genus	χ^2	df	P
Daphnia	2.91	3	0.406
Bosmina	7.89	3	0.048*
Asplanchna	0.89	3	0.829
Cyclops	0.35	3	0.951

* Statistically significant results.