

IMPACT OF NUTRIENT AND WATER LEVEL CHANGES ON
SUBMERGED MACROPHYTES ALONG A LATITUDINAL GRADIENT:
A PAN-EUROPEAN MESOCOSM EXPERIMENT

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SUBMERGED MACROPHYTES ALONG A LATITUDINAL GRADIENT:
PAN-EUROPEAN MESOCOSM EXPERIMENTS**

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ABSTRACT

IMPACT OF NUTRIENT AND WATER LEVEL CHANGES ON SUBMERGED MACROPHYTES ALONG A LATITUDINAL GRADIENT: A PAN-EUROPEAN MESOCOSM EXPERIMENT

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Ongoing climate warming will affect freshwater ecosystems worldwide. In addition to a temperature rise, increased precipitation and enhanced nutrient runoff is anticipated for Northern Europe, whereas decreased precipitation, decreased water levels and intensified drought conditions are expected for Southern Europe and the Mediterranean region. We conducted a controlled Pan-European mesocosm experiment in six countries along a latitudinal temperature gradient from Sweden to Greece to elucidate the effect of climate on macrophyte growth in shallow lakes with contrasting depths (shallow and deep) and nutrient levels (low and high). Physico-chemical variables, light attenuation and percent volume inhabited (PVI %) of submerged plants were sampled monthly during one season (May-November 2011). At the end of the experiment, macrophytes were harvested and dried. Water level changed only marginally (ranging from -3 to +8 cm) in northern countries; whereas an average of 46 cm and 83 cm water level decrease occurred in Turkey and Greece, respectively. Mean air temperature was highest in Greece (23.4 °C), followed by Turkey (18.7 °C). During the experiment, submerged macrophytes grew in all shallow mesocosms irrespective of nutrient treatments.). ANCOVA results indicated a significant effect of depth- nutrient and temperature-nutrient interactions on PVI%. High temperatures with low nutrient conditions gave rise to higher PVI% in shallow mesocosms which followed an increasing pattern from Sweden to Greece with an increase in mean air temperature. Shallow high nutrient

mesocosms also exhibited high PVI% in Turkey, likely due to decreased water level, but not in Greece where extreme water level drop was detrimental for macrophyte growth. We conclude that the combined effects of water level decrease and temperature increase stimulated macrophyte growth even under unfavourable light environment and high nutrient conditions. Nonetheless, an extreme water level decrease had negative effects on macrophyte growth by means of sun exposure, desiccation and eutrophication. Our results therefore indicate that global climate warming and eutrophication may negatively affect the development of macrophytes, if longer and intense drought periods become more common.

Keywords: climate change, macrophytes, mesocosm experiment

ÖZ

BESİN TUZU VE SU SEVİYESİ DEĞİŞİMİNİN SUIÇİ BİTKİLERİ ÜZERİNDEKİ ETKİSİNİN ENLEMSEL ÖLÇEKTE İNCELENMESİ: PAN-AVRUPA MEZOKOZM DENEYİ

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İklim değişikliği dünyadaki tatlısu ekosistemlerini tehdit etmektedir. Sıcaklık artışının yanı sıra, Kuzey Avrupa’da yağışlarda ve yağışlarla taşınan besin tuzu miktarında artış, Güney Avrupa ve Akdeniz bölgelerinde ise yağışlarda azalmayla kuraklığın artması beklenmektedir. İklim değişikliğinin suiçi bitkileri üzerindeki etkisini araştırmak için, İsveç’ten Yunanistan’a uzanan enlemsel gradyanda iki ayrı su seviyesi(Sığ: 1m, Derin: 2m) ve iki ayrı besin tuzu yoğunluğunda (düşük ve yüksek besin tuzu) kontrollü mezokozm deneyleri yapılmıştır. Deney boyunca mezokozmlardaki fiziko-kimyasal değişkenler, ışık geçirgenliği ve suiçi bitkisi kaplama yüzdesi (PVI%) aylık olarak ölçülmüştür. Deneyin sonunda, suiçi bitkileri toplanmış, kurutulmuş ve kuru ağırlıkları hesaplanmıştır. Kuzey Avrupa ülkelerinde su seviyesinde çok değişiklik görülmezken (yaklaşık -3cm - +8 cm), Türkiye ve Yunanistan’da sırasıyla 46 cm ve 83 cm su seviyesi düşüşü görülmüştür. En yüksek ortalama sıcaklıklar Yunanistan (23.4 °C) ve Türkiye’de (18.7 °C) kaydedilmiştir. Deney boyunca makrofitler, besin tuzu miktarına bakılmaksızın tüm sığ tanklarda büyümüştür. ANKOVA sonuçlarına göre, derinlik-besin tuzu ve sıcaklık-besin tuzu etkileşiminin PVI% üzerinde önemli etkisi olduğu gözlenip, sığ ve düşük besin tuzlu mezokozmlarda PVI% daha fazla bulunmuştur. Düşük besin tuzlu mezokozmlarda sıcaklığın İsveç’ten Yunanistan’a doğru artışıyla birlikte, PVI% da artış göstermiştir. Türkiye’de sığ yüksek besin tuzlu mezokozmlarda da yüksek PVI% gözlenmiştir. Bunun nedeni Türkiye’deki mezokozmlardaki su

seviyesi düşüşü olabilir. Fakat, aşırı su seviyesi ötrofikasyona ve kuraklığa neden olabildiği için, Yunanistan'da sığ yüksek besin tuzlu mezokozmlarda yüksek PVI% gözlenmemiştir. Sonuç olarak, su seviyesi düşüşü ve sıcaklık artışının yüksek besin tuzlu durumlarda dahi makrofitlerin büyümesini tetiklediği gözlenmiş, fakat aşırı su seviyesi düşüşünün ise olumsuz etkilere neden olabileceği saptanmıştır. İklim değişikliğiyle birlikte artan ötrofikasyonun, eğer kuraklık dönemleri artarsa makrofitleri kötü etkileyebileceği tahmin edilmektedir.

Anahtar Kelimeler: iklim değişikliği, makrofit, mezokozm deneyleri

To my family

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LIST OF ABBREVIATIONS

TP	Total Phosphorous
TN	Total Nitrogen
Chl <i>a</i>	Chlorophyll a
Kd	Light Attenuation Coefficient
PCA	Principal Component Analysis
PVI	Plant Volume Inhabited
SFTS	Space-for-time Substitution

CHAPTER 1

INTRODUCTION

1.1 Importance of Shallow Lakes

Freshwaters constitute a small portion (3%) of the world's water resources, rivers and inland waters making up only 0.02 % (Moss, 2010). Although they are small in proportion, they have quite important roles in structure and functioning of the ecosystems and biosphere. Freshwater species constitute 15% of all living animal species (Brönmark & Hansson, 2005). The ecosystem services provided by freshwater ecosystems include climate regulation, water purification, food, biodiversity, recreation and ecotourism etc. Therefore, freshwater ecosystems play enormous roles in the fate of modern human civilizations. However, since they are extremely sensitive to environmental changes, freshwater ecosystems are facing great threats with increasing human population, pollution, invasive species, over-exploitation and human-induced climate warming at the moment. This destruction of freshwater ecosystems does not only affect human civilizations now, but also intrinsic biological values of freshwaters. Freshwater biodiversity is decreasing far larger than any other terrestrial ecosystems (Dudgeon et al., 2006).

Research about freshwater ecosystems has focused mostly on deep lakes (Moss, 1998; Moss et al., 2005). However, shallow and small lakes make up the largest proportion of the lakes worldwide, defined as having average depth of 3 m and no stratification in summers. They have a large littoral zone with macrophyte beds, offering diverse habitats for zooplankton, fish and macroinvertebrates and having a significant structuring role in ecosystems (Moss, 1998; Scheffer, 1998). Also, they are very rich in biodiversity and provide most of the ecological services of the freshwaters. However, because they are small and shallow, they are more prone to be affected by the changes in the environment.

1.2 Alternative Stable States

For a long time, changes in environmental conditions had been assumed to cause gradual changes in ecosystems. Nonetheless, studies of many ecosystems including forests, coral reefs, terrestrial and freshwater ecosystems revealed that most responses to these changes are not gradual, but abrupt. According to alternative stable states theory, once a critical threshold has been surpassed sudden changes occur in ecosystems and it is difficult to restore the original ecosystem properties. Since they lack early warning signals, these changes in ecosystems are called catastrophic shifts (Scheffer et al., 2001). For instance, desertification of Sahara region was suggested as a catastrophic shift. These shifts also occur in lakes (Scheffer et al., 2003). Shallow lakes mostly exhibit two alternative states, a clear water with abundant aquatic vegetation and a turbid water state with high phytoplankton abundance (Scheffer et al., 1993).

Several ecological mechanisms are involved in alternative stable states, including both biotic and abiotic interactions. Nutrients, light availability and water level are among the abiotic factors, while food web interactions constitute the biotic interactions. In clear water state, aquatic vegetation promote water clarity, by providing habitat for zooplankton and macroinvertebrates and by competing with phytoplankton for nutrients and releasing alleopathic chemicals which inhibit growth of certain phytoplankton. On the other hand, increase in nutrients, turbidity and thus light limitation prevent growth of submerged vegetation, allowing phytoplankton to grow and to benefit from light and nutrients in turbid water state (Scheffer et al., 1993).

With increasing nutrient addition from catchment or by internal loading, shallow lakes have a tendency to change from clear water to turbid water (Moss, 1990). However, once a critical turbidity threshold is passed, mostly it is difficult to shift the system to the clear water state (Fig. 1.1). More strong nutrient reductions are necessary to change the system back.

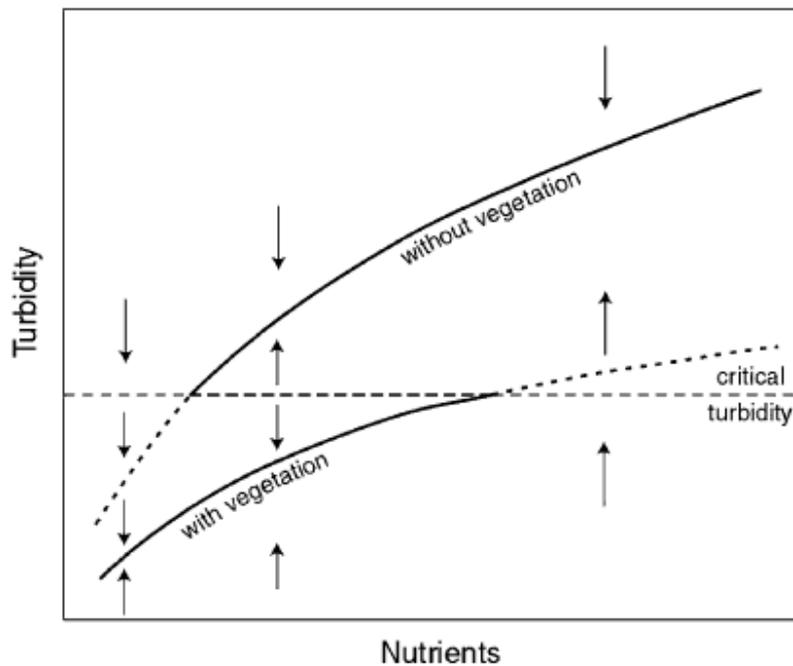


Figure 1.1 Alternative stable states theory, taken from Scheffer et al., 1993

These catastrophic shifts could result in destruction in functioning and structure of ecosystems. Therefore, it is necessary to identify early warning signals and forecast the threshold for the shift for management issues (Scheffer et al., 2001). Although there is evidence for alternative stable states evidences from long-term field data and modelling, experiments are needed to explore and take mitigation measures for catastrophic regime shifts (Scheffer et al., 2003).

1.3 Role of Submerged Macrophytes in Shallow Lakes

Macrophytes are highly important for the structure and functioning of shallow lakes (Moss, 1990; Scheffer et al., 1993; Jeppesen et al., 1997). They contribute to creating and maintaining a clear water state in temperate shallow lakes (Scheffer et al., 1993; Jeppesen et al., 1997; Blindow et al., 2000) through several stabilizing buffer mechanisms. They provide diverse habitats for fish, macroinvertebrates and birds and act as a refuge for zooplankton (Timms & Moss, 1984; Burks et al., 2002), thereby keeping phytoplankton abundance low. They decrease sediment resuspension (Barko & James, 1998; Madsen et al., 2001) and therefore, decrease release of nutrients from sediments (Søndergaard et al., 1992). They compete with

phytoplankton for light and nutrients by means of several alleopathic processes (Van Donk & Van de Bund, 2002). Furthermore, by providing organic carbon and surface for denitrifying microorganisms, they facilitate nitrogen removal in shallow lakes (Weisner et al., 1994).

Nonetheless, such positive roles of macrophytes may not be in action in warmer lakes due to aggregation of many small fish (Jeppesen et al., 2010; Brucet et al., 2013) within macrophyte beds (Meerhoff et al., 2007) resulting in reduced top-down control of zooplankton on phytoplankton. Thus, macrophytes might not act as an efficient refuge for zooplankton in warm temperate (González Sagrario & Balseiro, 2010), subtropical (Meerhoff et al., 2006, 2007) and Mediterranean shallow lakes (Tavşanoğlu et al., 2012). However, due to weak positive feedback mechanisms stabilizing buffer mechanisms of submerged macrophytes on water clarity are not well-defined in warm lakes (Bachmann et al., 2002; Iglesias et al., 2007; Castro, Marques & Gonçalves, 2007).

Also, submerged macrophytes have an important role in the implementation of European Union Water Framework Directive (WFD) that aims to achieve good ecological status in all freshwater ecosystems by 2015. They are one of the four biological variables used to reflect this good ecological status of lakes (Søndergaard et al., 2005; Dudley et al., 2013). With the loss of macrophytes, shallow lakes tend to lose their key ecosystem services including drinking water supply, biodiversity and recreational activities. Most of the restoration strategies aim at the return of macrophytes (Bakker et al., 2013). Knowing and understanding the factors affecting macrophyte communities make it possible to reconstruct destroyed and altered lake ecosystems to their reference states.

1.4 Factors Affecting Submerged Macrophytes

In addition to their important roles in ecosystems, macrophytes are also highly sensitive to environmental changes, either natural or anthropogenic. Nutrients through indirect effects, temperature and water level changes are of primary importance on abundance and distribution of macrophytes. However, these effects are different in different climatic conditions.

1.4.1 Nutrients

So far, many studies have revealed that nutrients influence the abundance and richness of macrophytes indirectly, by mediating periphyton and phytoplankton growth, and thus affecting under water light environment. In Denmark, many lakes have lost their macrophyte coverage in past 100 years because of increased nutrient enrichment causing increased phytoplankton biomass and decreased water transparency (Sand-Jensen et al., 2000). Growth of macrophytes could also be weakened due to increased nutrients via internal loading in both warm (Özen et al., 2010) and cold (Jeppesen et al., 2015) climates. So far, many studies revealed that growth of submerged plants could be weakened or they may even disappear due to shading by periphyton and increasing phytoplankton turbidity (Phillips et al., 1978; Gonzalez Sagrario et al., 2005; James et al., 2005; Romo et al., 2013). Particularly phosphorus and nitrogen are known to affect growth of macrophytes through indirect ways. For instance, a mesocosm experiment conducted in Denmark showed that macrophytes disappear when phosphorus and nitrogen concentration increase above certain levels (Gonzalez Sagrario et al., 2005). However, these nutrient concentrations differ across climates. Lower nutrient thresholds were reported in warmer than in colder regions (Romo et al. 2004, Kosten et al., 2009).

1.4.2 Temperature

Response of macrophytes to warming is complicated and could either be positive or negative. In warmer climates, submerged macrophytes may benefit from warmer temperatures and higher light intensity, by having higher growth rates which potentially increase their resistance to higher nutrient levels as they can grow faster to the surface (Scheffer et al., 1992; Romo et al., 2004; Kosten et al., 2009). Moreover, submerged macrophytes may take advantage of higher light availability caused by low periphyton biomass due to fish grazing (Meerhoff et al., 2007) and longer growing seasons (Rooney & Kalff, 2000). However, warming could cause disappearance of submerged macrophytes indirectly by intensifying eutrophication (Moss et al., 2011). Shifts to phytoplankton dominated turbid state and loss of macrophytes are anticipated with temperature increase in both north temperate (Mooij et al., 2005) and Mediterranean shallow lakes (Jeppesen et al., 2015).

1.4.3 Water Level Fluctuations

Response of macrophytes to water level fluctuations is unclear and dependent on different factors. Many studies indicate evident positive relationship between water level decrease and macrophyte growth in different climatic regions (Blindow et al., 2002; Coops, Beklioglu & Crisman, 2003; Beklioğlu, Altınayar & Tan, 2006; Romo et al., 2004; Tan & Beklioğlu, 2006; Beklioğlu et al., 2007; Rip et al., 2007), as water level decline could result in increased light availability for submerged vegetation and may cause expansion of the littoral zone (Coops et al., 2003; Beklioglu et al., 2006; Beklioglu & Tan, 2008; Kosten et al., 2009; Stefanidis & Papastergiadou, 2013). Recent mesocosm experiments from Turkey (Özkan et al., 2010; Bucak et al., 2012) and a long term monitoring study from Greece (Stefanidis & Papastergiadou, 2013) showed that water level decline caused by high evapotranspiration in summer compensates the indirect negative effects of nutrient loading on submerged macrophytes; despite high nutrient concentrations and low water clarity, macrophyte growth was higher due to a notable water level decline. On the other hand, there are several studies showing that growth of macrophytes

could be negatively affected by water level reduction. Extreme water level decline and drought conditions could destabilize growth of macrophytes by means of disruption of the littoral zone (Blindow 1992; Blindow et al., 1993; Beklioğlu et al., 2006). During low water level conditions, macrophytes could also be affected by decreased water transparency (Barone et al., 2010; Bresciani et al., 2012, Jeppesen et al., 2015), increased sediment exposure (Thomaz et al., 2006), increased nutrient concentrations (Özen et al., 2010) and increased salinity (Nielsen & Brock, 2003; Brock & Nielsen, 2005).

1.5 Climate Change Effects on Shallow Lakes

Climate warming is expected to affect freshwater ecosystems all over the world. Regional climate models have predicted warmer winter temperatures and higher precipitation in Northern Europe (IPCC, 2014), which would possibly enhance surface runoff, cause higher nutrient loading of phosphorus (P) and nitrogen (N) to the lakes and trigger eutrophication (Jeppesen et al., 2011). In shallow lakes, high risk of shifts to turbid water state is anticipated due to loss of macrophytes (Jeppesen et al., 2009; 2011).

Moreover, increased summer temperatures and evaporation, with significant decrease in precipitation have been predicted for central and southern Europe, especially for Mediterranean region (Giorgi, 2006; Alcamo et al., 2007; Giorgi & Lionello, 2008; Erol & Randyr, 2012; IPCC 2014), resulting in more intense and prolonged drought periods, while substantially reducing surface runoff (Milly et al., 2005) and external nutrient loading (Jeppesen et al., 2009). Therefore, together with the intense anthropogenic water use, these dry periods are expected to dramatically change lake hydrology, especially water level fluctuations in the Mediterranean region which play a significant role in ecology of shallow lakes especially in macrophyte growth (Coops et al. 2003; Beklioğlu et al. 2006; Papastergiadou et al. 2010; Stefanidis & Papastergiadou, 2013).

1.6. Mesocosm Experiments with Space-for-time Substitution Approach

Experiments are increasingly used in ecology in order to reveal complex ecosystem responses. They are only simple representation of real ecosystems if conducted in small scales. However, if experiments are carried on large scales, they could be quite realistic while representing natural ecosystems. Mesocosms are among frequently used experimental systems in freshwater ecology. They are basically enclosures containing main constituents of lake ecosystems and mimic the responses of natural system to experimental manipulations. Mesocosm experiments offer great opportunity to observe the gradual changes in the system and allow us investigate the effects clearly. Despite lacking complex interactions and can only explain short term responses, they can be replicated and controlled easily (Stewart et al., 2013; Jeppesen et al., 2014).

In addition to experiments, space-for-time substitution (SFTS) is a commonly used method to compare similar ecosystems in different climates (Jeppesen et al., 2014). Due to the absence of long-term ecological data, SFTS approach allows us to understand possible long term consequences of climate change. So far, several studies using SFTS approach revealed the effects of climate change in lake ecosystems (Gyllström et al. 2005; Meerhoff et al, 2007; Kosten et al., 2009). This approach is high in spatial resolution and it is quite predictive for adaptation of communities, though it disregards the importance of local geology, biogeography and land use (Meerhoff et al., 2012; Jeppesen et al., 2014).

However, single approaches are not sufficient to understand natural ecosystems completely, since they have some advantages and disadvantages. Also, because shallow lakes are complex ecosystems, it is difficult to observe the changes with climate change in natural ecosystems. By combining SFTS with controlled mesocosm experiments, these undesirable effects of locality can be minimized and ecosystem responses and complexity can be better elucidated (Woodward et al., 2010). This combination of methods helps us to compare controlled experiments in different climatic regions.

1.7 Aim of the Study

So far, few studies have focused on macrophytes in shallow lakes along a latitudinal climatic gradient (Heino, 2001; Chambers, 2008; Kosten et al. 2009; Lauridsen et al., 2015), and little is known on mechanisms controlling the growth of aquatic vegetation along a latitudinal gradient found in the literature (Meerhoff et al., 2012). We conducted a controlled Pan-European mesocosm experiment with two water levels (shallow and deep) and two nutrient levels (low and high) in six countries along a latitudinal temperature gradient from Sweden to Greece to elucidate the effect of climate on macrophyte growth in shallow lakes with contrasting depth and nutrient levels. We hypothesized that higher macrophyte growth would occur in shallow, low nutrient mesocosms than in high nutrient mesocosms due to high light availability and less macrophyte growth in deep mesocosms at both low and high nutrient levels. However, indirect negative effects of nutrients would be weaker in warmer southern countries especially in shallower mesocosms reflecting low water levels.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Sites

Mesocosm experiments were carried out simultaneously in 6 countries along a latitudinal gradient: Sweden, Estonia, Germany, Czech Republic, Turkey and Greece (Fig. 2.1, Table 2.1). The lakes for the experiment were selected following certain criteria in Table 2.2 (Landkildehus et al., 2014). These criteria are set in order to display natural properties of shallow lakes in Europe. The experiment lasted six months from May 2011 to November 2011 and all samplings and additions were conducted according to a highly synchronized protocol followed by all countries to guarantee comparability of the results from separate experiments.

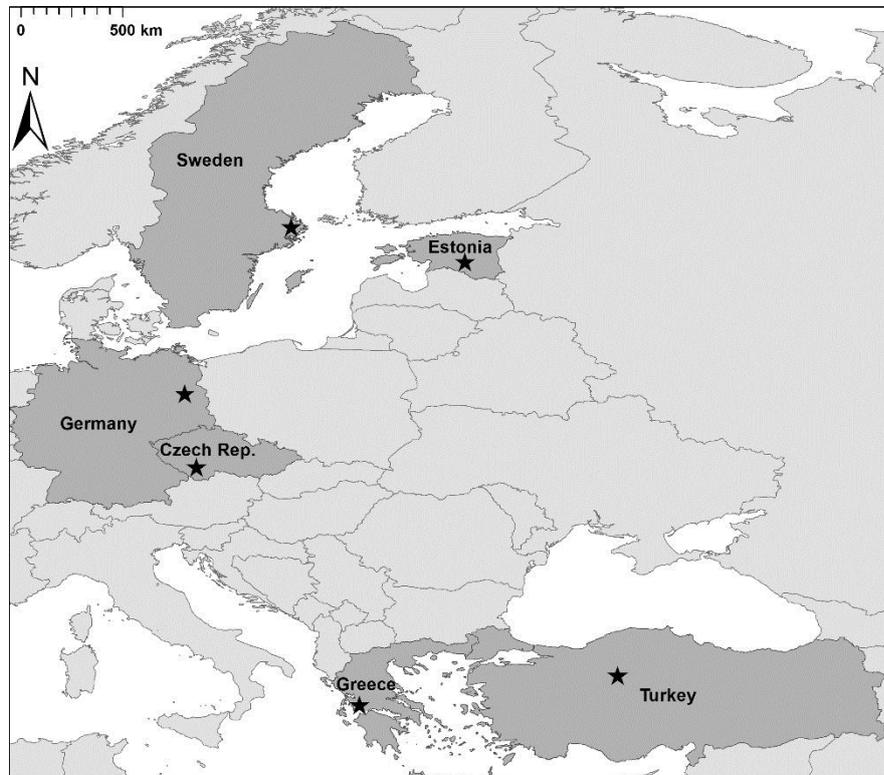


Figure 2.1 Geographical location of experimental sites.

Table 2.1 General information about experimental sites during the mesocosm experiment (May-November 2011)

Country	Coordinates	Altitude (m a.s.l.)	Mean air temperature (°C)	Total precipitation (mm)
Sweden	59°49'59"N 18°33'55"E	11	14.6	271
Estonia	58°12'17" N 26°06'16" E	35	14.9	298
Germany	52°26'0" N 13°39'0" E	32	16.9	431
Czech Republic	49°09'14"N 14°10'11"E	395	15.3	401
Turkey	39°52'38" N 32°46'32" E	998	18.7	212
Greece	38°33'40" N 21°22'10" E	16	23.4	252

Table 2.2 Selection criteria for lakes

Variables	Criteria
Mean depth	< 4 m
Nutrient concentration	< 25 µg l ⁻¹ TP
Total alkalinity (TA)	1 < TA < 4 meq l ⁻¹
Salinity	< 1 ‰
Colour	< 20 mg Pt-Co l ⁻¹

2.2 Experimental Set-up

The experiments followed a $2 \times 2 \times 4$ factorial design with two water level treatments (shallow: 1 m height, deep: 2 m height), two nutrient levels (aiming at high: $200 \mu\text{g l}^{-1}$ total phosphorus (TP) and 2.0 mg l^{-1} total nitrogen (TN), low: $25 \mu\text{g l}^{-1}$ TP and 0.5 mg l^{-1} TN) representing eutrophic and oligotrophic conditions, and 4 replicates for each treatment. Treatments were represented as shallow and high nutrient level (SH), shallow and low nutrient level (SL), deep and high nutrient level (DH), deep and low nutrient level (DL).

The experimental set-up consisted of 16 mesocosms which were closed enclosures made of fiberglass with a diameter of 1.2 m, and different heights of 1.2 and 2.2 m (Fig.2.2). All enclosures were made by same manufacturer (Armaplast Polyester San. ve Tic. Ltd., Gebze, Turkey) and transported to other countries. They were attached to a floating pontoon bridge equipped with a wooden boardwalk and anchored at the bottom. Fruit nets were placed above the enclosures in order to avoid interference of birds. The mesocosms were placed in a randomised block design but followed the same pattern in all the countries (Fig. 2.3 & 2.4, Table 2.3).

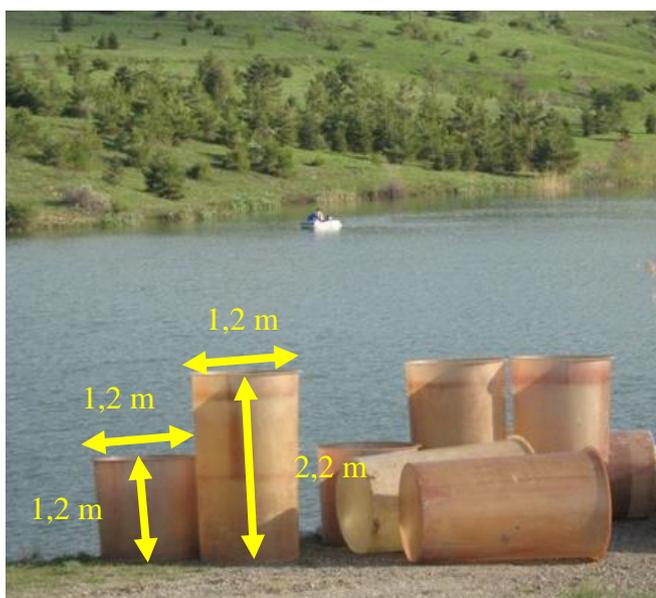


Figure 2.2 Shallow and deep mesocosms (Taken from METU Limnology Laboratory Archive)

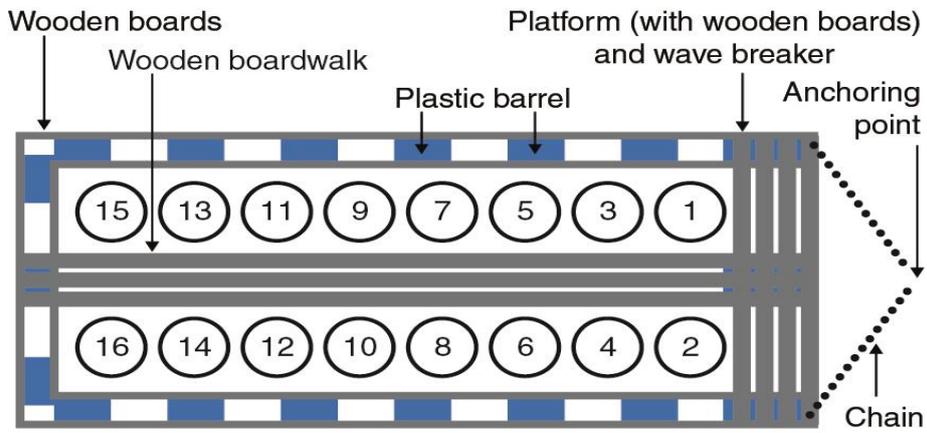


Figure 2.3 Experimental set-up (taken from Landkildehus et al., 2014)

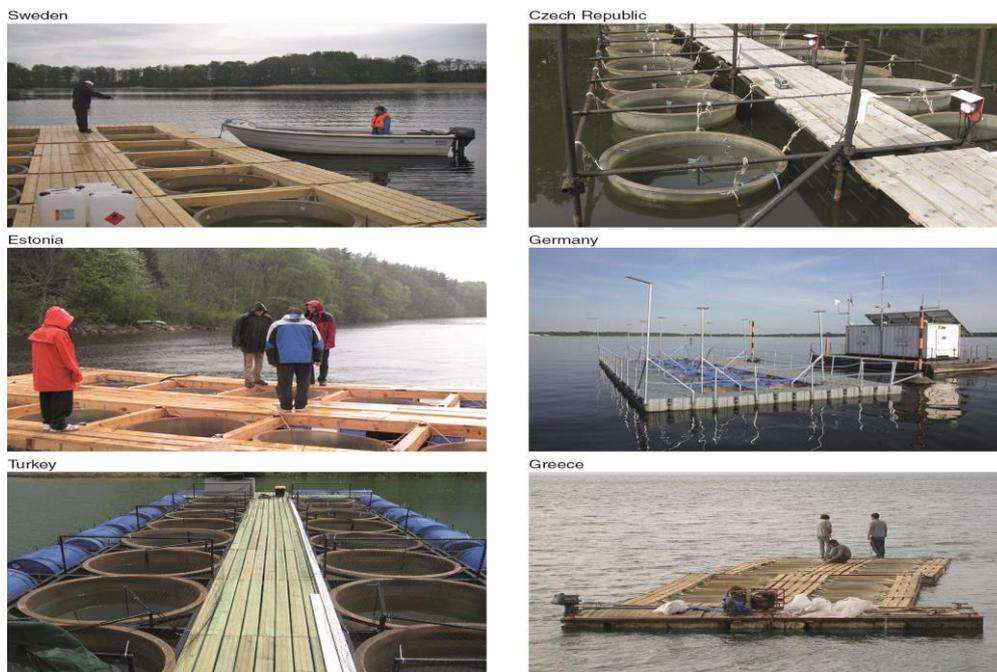


Figure 2.4 Experimental set-up in all countries

Table 2.3 Random distribution of the mesocosms in all countries

Enclosure number	Depth	Nutrient	Enclosure name
1	Shallow	High	SH 1
2	Deep	High	DH 1
3	Shallow	High	SH 2
4	Deep	Low	DL 1
5	Shallow	Low	SL 1
6	Shallow	High	SH 3
7	Deep	High	DH 2
8	Shallow	Low	SL 2
9	Shallow	High	SH 4
10	Deep	Low	DL 2
11	Shallow	Low	SL 3
12	Deep	High	DH 3
13	Deep	High	DH 4
14	Deep	Low	DL 3
15	Deep	Low	DL 4
16	Shallow	Low	SL 4

After the experimental set-up was formed, firstly a 10 cm sediment layer consisting of 90% washed sand (diameter < 1 mm) and 10% sediment taken from a nearby oligo-mesotrophic lake was added to the mesocosms once large particles were filtered from 10 mm mesh. Before the addition, the muddy part of the sediment layer was equilibrated to the desired initial nutrient levels (25 $\mu\text{g l}^{-1}$ TP in the low nutrient mesocosms and 200 $\mu\text{g l}^{-1}$ TP in the high nutrient mesocosms) in the laboratory for two months prior to the start of the experiment for eliminating the risk of sediment acting as either a source or a sink during the experiment. This sediment equilibration process lasted from winter of the previous year to the start of the experiment. After the enclosures were filled with 10 cm sediment, 20-50 cm water with low (25 $\mu\text{g l}^{-1}$ TP) or high (200 $\mu\text{g l}^{-1}$ TP) nutrient concentrations were added. Concentrations were controlled periodically to ensure targeted starting conditions, allowing $\pm 25\%$ deviation (Landkildehus et al.,2014).

After sediment addition, filtered water from the oligotrophic lakes where the mesocosms were installed, was added to the mesocosms in all countries, with the exception of Germany and Czech Republic where tap water was used as TP concentrations of their lake water were higher than $25 \mu\text{g l}^{-1}$ TP. Aquarium pumps were used in order to fully mix water in the mesocosms.

2.3 Inoculations & Nutrient loading

To achieve standard starting conditions in all countries, mixed plankton and sediment inocula collected from 5 local lakes (with a range of $25\text{-}200 \mu\text{g l}^{-1}$ TP gradient) were added to the mesocosms four days after the set-up in order to allow for sediment suspension. Five vertical net hauls by plankton net with a diameter of 20 cm and mesh size of $50 \mu\text{m}$ were obtained from 5 local lakes and pooled in each country. Mixed with lake water in 5 L containers, 1 L subsample of plankton inocula were added to mesocosms. Moreover, 1 L of 5 L sediment mix from same 5 local lakes were sieved through 10 mm mesh to remove fish eggs or mussels and also inoculated to each mesocosm (Landkildehus et al., 2014).

Four days after sediment and plankton inoculation, macrophytes and fish were added to the mesocosms. In all countries watermilfoil (*Myriophyllum spicatum*) was collected and kept cool until transplantation (Fig. 2.5.a). Watermilfoil was selected because it is the common dominant submerged macrophyte in all countries. In Germany, the plants were collected during the autumn of the previous year and stored in order to guarantee the availability of water milfoils at the beginning of the experiment. However in other countries, plants were collected just before starting the experiment. Before planting, the macrophytes were left in carbonated water for 15 minutes to eliminate invertebrates and snails. Five to ten cm long apical shoots of 8 plants were planted evenly in the central zone (diameter 0.5 m) of each mesocosm. Stones, weighing 5 g, were attached to the plants so as to allow them to reach the sediment. 4-20 grams of fish biomass were stocked in each mesocosm. Three-spined sticklebacks, *Gasterosteus aculeatus* L., were added to the mesocosms with a 1:1 sex ratio (six individuals) except in Sweden and Greece,

where roach (two individuals) (*Rutilus rutilus* L.) and western mosquito fish (six individuals) (*Gambusia affinis* Baird and Girard) respectively had to be used since transfer of species was not legal in Sweden and catching sticklebacks in Greece was difficult. (Landkildehus et al., 2014) (Fig. 2.5.b) It is acknowledged that these fish species prefer similar food sources as three-spined sticklebacks (Hynes 1950; Offill & Walton 1999). If any fish was found dead during the experiment, new fish was stocked back to the enclosures.

After establishing similar starting conditions, dissolved mixtures of calcium nitrate ($\text{Ca}(\text{NO}_3)_2$) and disodium phosphate (Na_2HPO_4) were added to the mesocosms monthly as P and N sources with a ratio of 1:20 in order to achieve the aimed nutrient levels. The loading rates of added nutrients were 5.1 mg P and 102 mg N in shallow low nutrient tanks (SL), 81.6 mg P and 1632 mg N in shallow high nutrient tanks (SH); 10.8 mg P and 216 mg N in deep low nutrient tanks (DL) and 172 mg P and 3440 mg N in deep high nutrient tanks (DH). Approximate amounts of calcium nitrate and disodium phosphate were recalculated for additions.



a. European watermilfoil (*Myriophyllum spicatum*). Taken from http://en.wikipedia.org/wiki/Myriophyllum_spicatum



b. *Gasterosteus aculeatus* L., *Gambusia affinis* Baird and Girard, *Rutilus rutilus* L.(from left to right). Taken from <http://www.fishbase.org/>

Figure 2.5 Macrophyte (a) and fish species (b) which were added to the mesocosms

2.4 Sampling and Laboratory Analysis

Sampling events started after the addition of macrophytes and fish. For determining of physico-chemical and biological variables, water samples were taken monthly from all mesocosms, with a tube sampler covering whole water column at 3 different positions: 30, 60 and 90 cm from the mesocosm wall. Water samples were then pooled, mixed and subsamples were taken for analysis of different parameters. Water samples were kept frozen until analysis for TP, TN and chlorophyll-a (Chl-*a*), which were determined using standard methods in each country. Water

temperature was determined monthly *in situ* using a multi-probe tool, together with water level change measurements.

Since Secchi depth measurements were not conducted in all countries, correlation analysis was performed in order to check the eligibility of the K_d (light attenuation coefficient) for the measure of light availability. In countries where Secchi depth was measured (CZ, GER, TR, GR), Secchi depth was found to be negatively correlated with K_d (Spearman correlation coefficient= -0.318, $p = 0.0134$, $n=60$). Therefore, light attenuation coefficient (K_d) was used as a measure of light availability.

Light attenuation (underwater photosynthetically active radiation, PAR) was measured in each mesocosm monthly with a light meter. The measurements were conducted at midday (between 11 a.m. and 1 p.m.) in every 10 cm along the water column until the light decreased to 10% of the incoming light. The diffuse attenuation coefficient of light (K_d) was calculated according to Beer-Lambert Law as:

$$K_d = \frac{\ln\left(\frac{E_{z_i}}{E_{z_{i+1}}}\right)}{z_{i+1} - z_i}$$

where E_{z_i} is the PAR measurement at z_i th depth and $E_{z_{i+1}}$ is the PAR measurement at z_{i+1} th depth measured downward. K_d was computed by calculating the light attenuation coefficient by layers and averaging it over depth. Subsequently, mean K_d for June to August were calculated from these values. Since there was little water left in shallow mesocosms of Greece, light attenuation measurements were not performed in Greece after August. Also, because of the technical problems in data loggers of Estonia, no light measurements were found in Estonia between August and September.

Macrophyte coverage for each mesocosm were determined monthly. Presence of macrophyte species other than *M. spicatum* were also recorded during each sampling. Macrophyte coverage was estimated by visual observation. The following scale was used to estimate percent macrophyte coverage; 0: no plants, 1: 0-5%, 2: 5-25%, 3: 25-50%, 4: 50-75%, 5: 75-95% and 6: 95-100%. Each coverage measurement was first changed to the scales mentioned above and then these numbers were converted to percentage by taking the mid-values of percentages. Furthermore, average height of the plants and water depth were also recorded during each sampling to calculate percent plant volume inhabited (PVI %) by using the following formula (Canfield *et al.*, 1984):

$$PVI(\%) = \frac{\% \text{ coverage} \times \text{average plant height}}{\text{water depth}}$$

Since separate coverage for each species was not obtained during the course of the experiment to avoid disturbance (only biomass measurements at the last day of experiment), all PVI (%) calculations were based on total coverage of all macrophytes and filamentous algae combined.

At the end of the experiment, aquatic vegetation in all mesocosms were harvested and dried at 60°C for 24 hours for calculation of macrophyte and, if present, of filamentous algae dry weight. Macrophyte biomass per area was calculated by dividing measured weight with surface area of the mesocosms. Separate dry weight data for macrophyte species were also available in all countries except Greece. Four mesocosms (one deep low nutrient, two deep high nutrient and one shallow high nutrient enclosures) in Germany were excluded from the analyses since they were overflowed or sank during the experiment.

Air temperature and precipitation measurements were taken from meteorological station on Lake Erken in Sweden, on Müggelsee in Germany, Centre for Limnology of the Estonian University of Life Sciences in Estonia, climatic station at Rimov Reservoir in Czech Republic, Middle East Technical University Physics Department and Turkish State Meteorology Service in Turkey and Hellenic National Meteorological Service in Greece.

2.5 Statistical Analyses

Time-weighted averages of all physico-chemical variables and PVI% were calculated according to the formula used by Hansson et al. (2004) and Stephen et al. (2004a, b). Values that were multiplied by the number of the sampling events were summed and divided by sum of the sampling events according to the following formula:

$$W_A = \frac{[(1 \times V_1) + (2 \times V_2) + \dots + (t \times V_t)]}{1 + 2 + \dots + t}$$

where W_A is weighted average and V_t is the variable at sampling time t . Time-weighted averages were used in all of the analyses. By this approach, data points in later sampling events had higher importance in the average, allowing experimental set-up to settle and acclimatize. Weighted averaging also ensures that dataset meet the requirements of the conducted parametric tests (Stephen et al., 2004).

R version 2.15.1 (R Core Team, 2012) was used to conduct ordination analysis (principal component analysis-PCA) in order to elucidate differences between treatments and measured variables, TP, TN and water level. Analysis of similarity (ANOSIM) was conducted to test the significant differences between treatments (Clarke, 1993).

Mean TP, TN, Chl-*a*, Kd, PVI% and macrophyte biomass were tested by analysis of covariance (ANCOVA) using the Proc Mixed method in SAS (SAS Institute Inc., Cary, NC, USA). In order to meet the assumptions of ANCOVA, transformations (ln and square root) were made, when necessary. Variables from all mesocosms were analyzed together, taking nutrient and depth as fixed factors. Air temperature was highly correlated with water temperature across all the data ($r=0.98$, $p<0.001$), thus the mean air temperature of countries was used as a covariate in the model, instead of water temperature. The results of the ANCOVA are given for fixed factors, cofactor and the interaction effects for both shallow and deep mesocosms. Since ANCOVA only looks for the effects of fixed factors (categorical variables), generalized linear models (GLM) with Gaussian distribution family were also used to assess the effects of environmental parameters such as measured nutrient concentrations, Kd and water level measurements on PVI % and macrophyte biomass. Collinear variables ($r>0.6$) were removed from the analysis, and regression was performed stepwise by removing the weakest predictor in order to assess the best predictors of the model.

CHAPTER 3

RESULTS

3.1 Physicochemical Variables

During the experiment, mean air temperature (May – October) was highest in Greece (23.4 °C), followed by Turkey, Germany, Czech Republic, Estonia and Sweden (Fig. 3.1). Differences in temperature was significant (one-way ANOVA, $p < 0.05$). Pairwise comparisons between countries revealed that while temperature in Greece was significantly different from Sweden, Estonia and Czech Republic ($p = 0.02$, $p = 0.02$ and $p = 0.03$, respectively), it did not differ from Germany and Turkey significantly.

The highest water level decline was observed in Greece (approximately 83 cm), followed by Turkey (46 cm); while only small changes occurred in the other countries (Fig. 3.1).

Break point analysis was conducted by using piecewise regression with mean air temperature and water level measurements in order to group the countries according to their temperature measurements. It was observed that Turkey and Greece were classified as warmer countries, while the other four countries were colder.

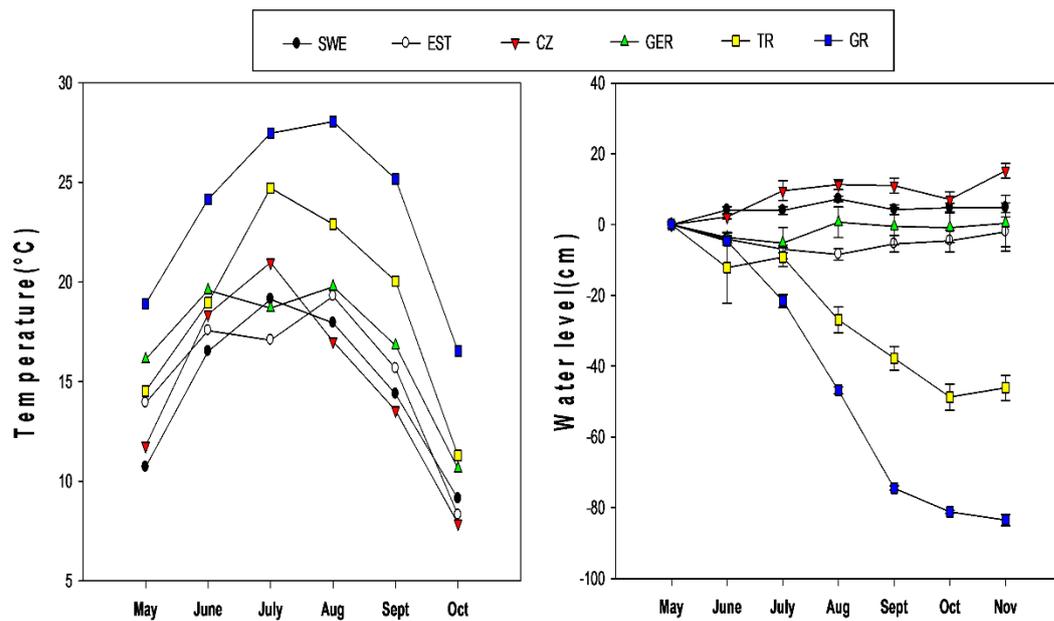


Figure 3.1 Changes in mean monthly air temperature (°C) (left) and mean monthly water level (cm) in mesocosms (right) throughout the experiment in all countries. Error bars show standard deviation.

Results of the ordination analysis was shown in Figure 3.2. The first two axes of the principal component analysis (PCA) explained 89% of the observed variance among the treatments. For the first axis, TN and TP contributed most importantly and positively. For axis 2, water level had the largest positive contribution. (Table 3.1) All deep mesocosms were distinguished by higher water level, except deep mesocosms of Turkey and Greece. Likewise, all shallow mesocosms had lower water level. Low nutrient treatments were characterized by low TP and TN for both shallow and deep mesocosms, except low nutrient treatment in Germany which had high TP and TN. The ANOSIM test subsequent to PCA showed that the all treatments (SL, SH, DL, DH) differed significantly in terms measured treatment variables ($p < 0.001$, $R = 0.49$).

Table.3.1 Principal component loadings.

	PC1	PC2
TP	1.45	-0.48
TN	1.51	-0.14
Water level	0.58	-1.55

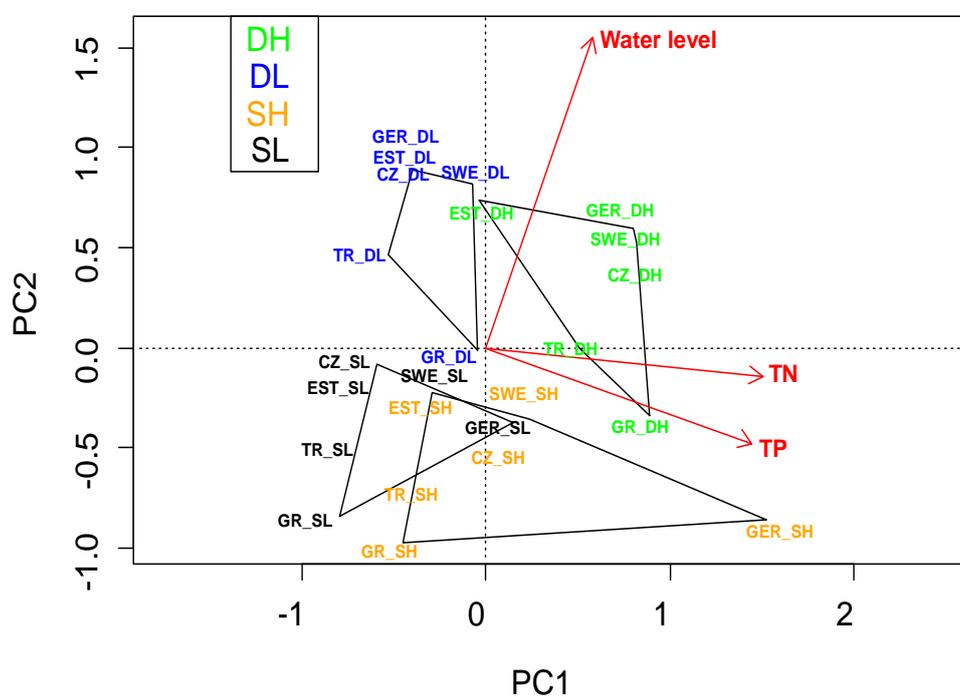


Figure 3.2 PCA plot of measured treatment variables in experimental mesocosms: water level TP, TN, All variables were standardized (z-transformation) before analysis. SL, shallow low nutrient treatment; SH, shallow high nutrient treatment; DL, deep low nutrient treatment; DH, deep high nutrient treatment. SWE: Sweden, EST: Estonia, CZ: Czech Republic, GER: Germany, TR: Turkey, GR: Greece.

The results indicated that both TP and TN concentrations were significantly affected by nutrient (ANCOVA, $p < 0.001$ for both, Table 3.2) and by temperature-depth interaction (ANCOVA, $p < 0.05$, $p < 0.001$, respectively, Table 3.2). Mean nutrient concentrations in each treatment for all countries are shown in Figures 3.3 & 3.4. Effect of nutrient treatment on TP and TN concentrations clearly showed that nutrient loading throughout the experiment reached the high and low nutrient levels. Germany had the highest TP in shallow mesocosms with a high variance among replicates. For TN, highest mean concentrations were observed in high nutrient mesocosm of Germany, and in deep high nutrient mesocosms of Greece.

Table 3.2 Summary of ANCOVA results with depth and nutrients as fixed factors; temperature as covariate.

Effect	TP ($\mu\text{g l}^{-1}$)	TN (mg l^{-1})	Chl-<i>a</i> ($\mu\text{g l}^{-1}$)	K_d (m^{-1})	PVI (%)	Macrophyte DW (g m^{-2})
Depth	*	*	ns	ns	**	***
Nutrient	***	***	***	ns	ns	ns
Depth*Nutrient	ns	ns	*	ns	*	ns
Temp	ns	ns	ns	***	***	ns
Temp*Depth	**	***	ns	ns	ns	**
Temp*Nutrient	-	-	-	ns	*	ns

DW, dry weight; ns, not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

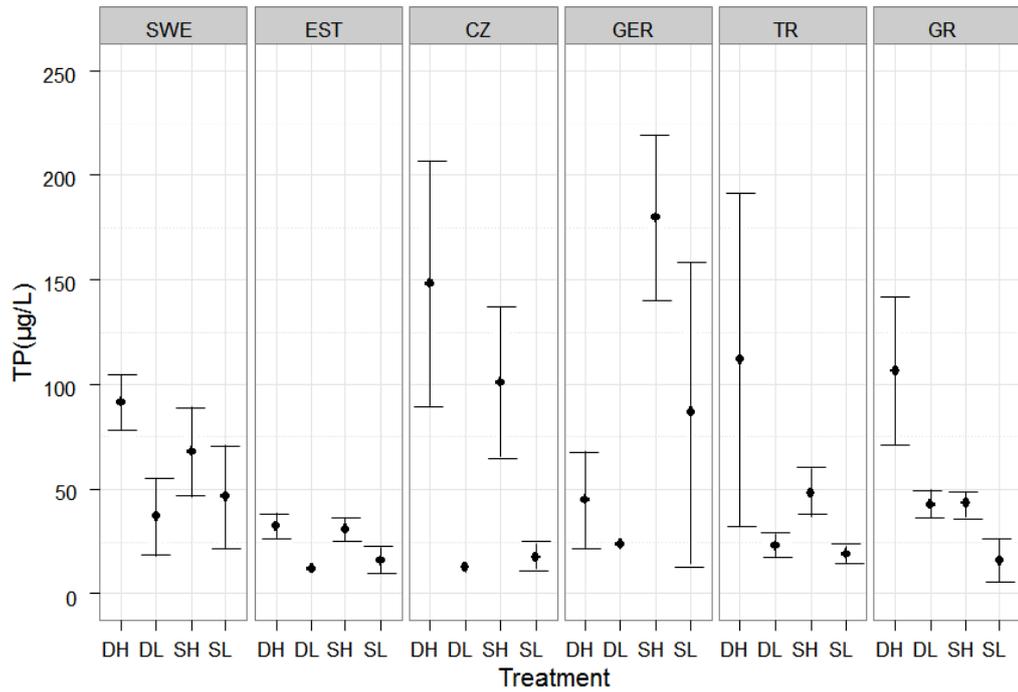


Figure 3.3 Mean total phosphorus (TP) ($\mu\text{g/L}$) in each treatment in all countries (Abbreviations of countries, SL, DH, SL and DL, explained at Fig. 3.2). Countries are ordered according to increasing temperature from left to right. Error bars show standard deviation.

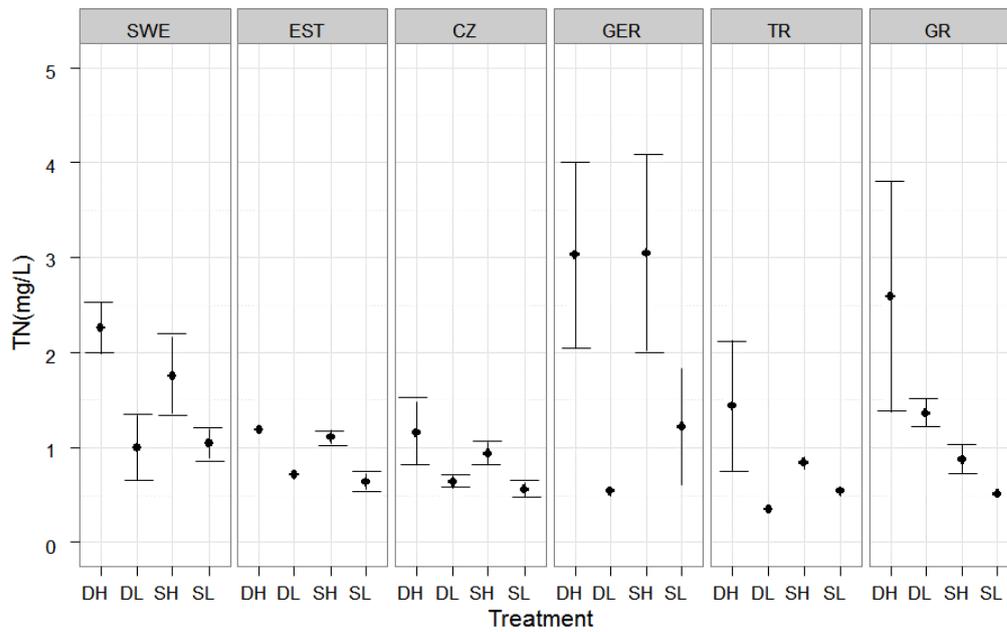


Figure 3.4 Mean total nitrogen (TN) (mg/L) in each treatment in all countries (Abbreviations of countries, SL, DH, SL and DL, explained at Fig. 3.2. Countries are ordered according to increasing temperature from left to right. Error bars show standard deviation.

Only temperature had a significant effect on light attenuation coefficient (K_d) (ANCOVA, $p < 0.001$, Table 3.2). Among all mesocosms (from June until August) K_d was generally found to be higher in Germany and Greece, while it was lowest in Turkey (Fig.3.5).

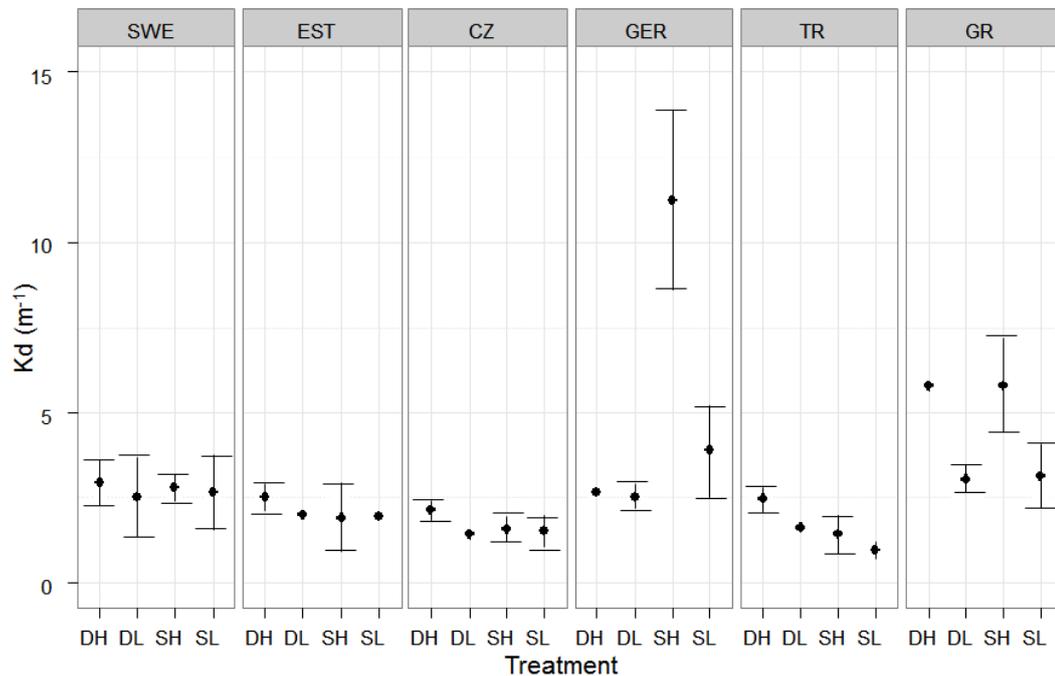


Figure 3.5. Mean light attenuation coefficient (K_d) in June-August in each treatment in all countries (Abbreviations of countries, SL, DH, SL and DL, explained at Fig. 3.2). Countries are ordered according to increasing temperature from left to right. Error bars show standard deviation.

3.2. Biological Variables

We found a marginally significant effect of depth and nutrient interaction on Chl-*a* (ANCOVA, $p=0.043$, Table 3.2). Deep and high nutrient mesocosms had higher Chl-*a* for all countries, except Germany where shallow high nutrient mesocosms had higher Chl-*a* concentrations (Fig. 3.6). The highest K_d followed the Chl-*a* concentration pattern that was observed in shallow German mesocosms and deep high nutrient mesocosms of Greece. It was strongly correlated with Chl-*a* concentrations ($n=47$, $r=0.67$, $p<0.001$).

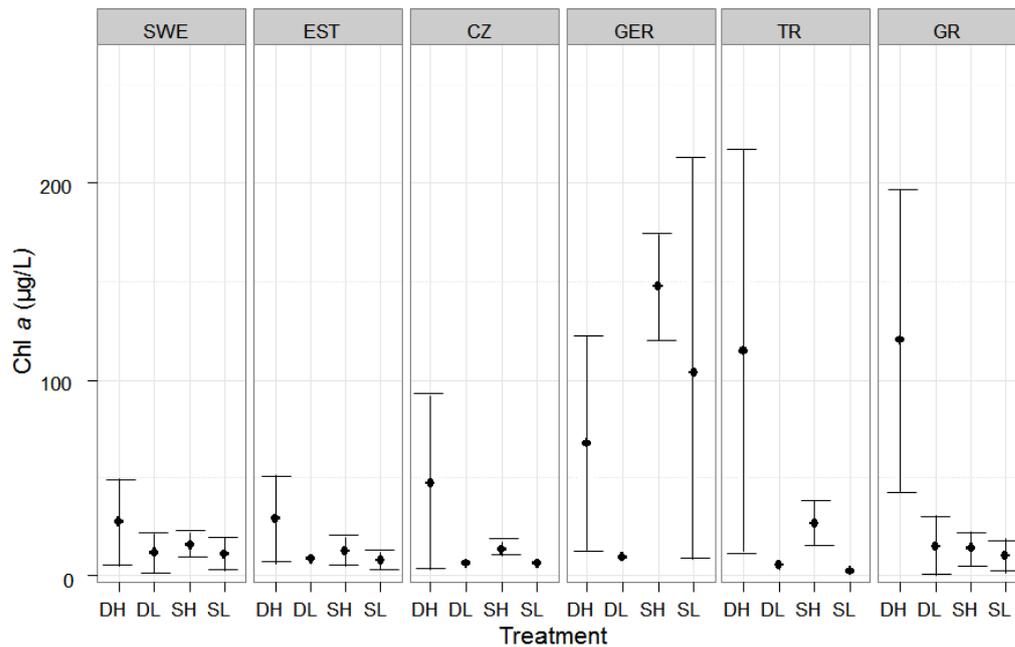


Figure 3.6 Mean chlorophyll a in each treatment in all countries (Abbreviations of countries, SL, DH, SL and DL, explained at Fig. 3.2). Countries are ordered according to increasing temperature from left to right. Error bars show standard deviation.

During the experiment, submerged macrophytes grew in all shallow mesocosms irrespective of nutrient treatments. PVI reached over 40% in SL mesocosms of Turkey and Greece. While Germany and Czech Republic had approximately 20% PVI, while in Sweden and Estonia it was less than 15%. The greatest growth in SH mesocosms was observed in Turkey, followed by Sweden and Greece. Macrophyte growth in DL mesocosms (at most 24% PVI) was mostly found in Greece and Germany. However, other countries did have a negligible growth in DL treatment. Only Greece and Turkey had macrophyte growth in DH treatment, but no growth was observed in the other countries (Fig. 3.7).

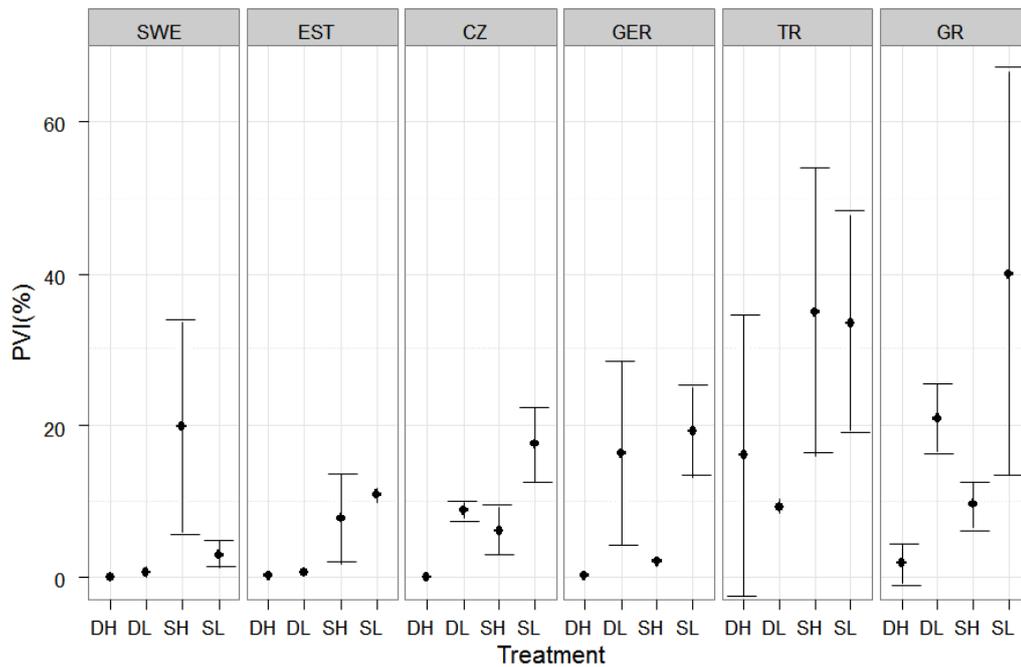


Figure 3.7 Weighted mean PVI in each treatment in all countries (Order of countries, SL, DH, SL and DL, explained at Fig. 3.2). Countries are ordered according to increasing temperature from left to right. Error bars show standard deviation.

According to ANCOVA results, depth and nutrient interaction had a significant effect on PVI% ($p < 0.05$), whereas nutrients alone had no effect. The interaction between temperature and nutrient had also significant effect ($p < 0.05$, Table 3.2) on PVI%. Higher PVI% was observed in shallow-low nutrient tanks. In addition, high temperatures with low nutrient conditions gave rise to higher PVI% in shallow mesocosms which followed an increasing pattern from Sweden to Greece with an increase in mean air temperature (Fig. 3.7). However, the effect of temperature and nutrient interaction was observed to a lesser extent in deep mesocosms.

Dry weights of harvested macrophytes at the end of the experiment varied among countries that followed the PVI% pattern. Depth and temperature interaction had a significant effect on macrophyte total biomass (ANCOVA, $p < 0.005$, Table 3.2), biomass being higher in shallow mesocosms of warmer countries. Both nutrient treatments of shallow and only low treatments of deep mesocosms had higher dry weight, with the exception of Greece (Fig. 3.8). However, macrophyte biomass was low in deep high nutrient mesocosms. In addition to planted dominant species *Myriophyllum spicatum*, other taxa such as *Najas marina*, *Chara* sp. and filamentous algae were also observed during samplings as well as at the end of the experiment in Estonia, Germany and Turkey. While Sweden and Czech Republic did not have any other taxa than the added species; *Chara* sp. was found mostly in shallow mesocosms of Estonia, Germany and Turkey. Turkey and Germany also had *Chara* sp. in both shallow and deep low nutrient mesocosms (Fig. 3.9).

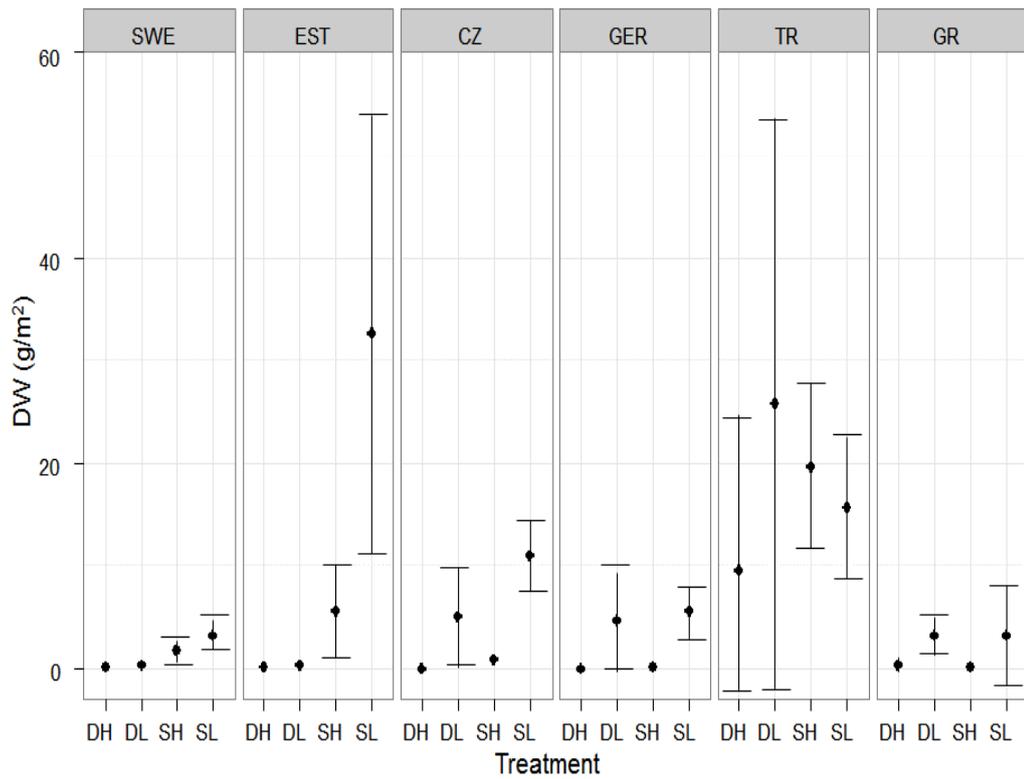


Figure 3.8 Boxplots of macrophyte dry weight (g/m²) at the end of the experiment in the different treatments (Abbreviations of countries, SL, DH, SL and DL, explained at Fig. 3.2). Countries are ordered according to increasing temperature from left to right. Error bars show standard deviation.

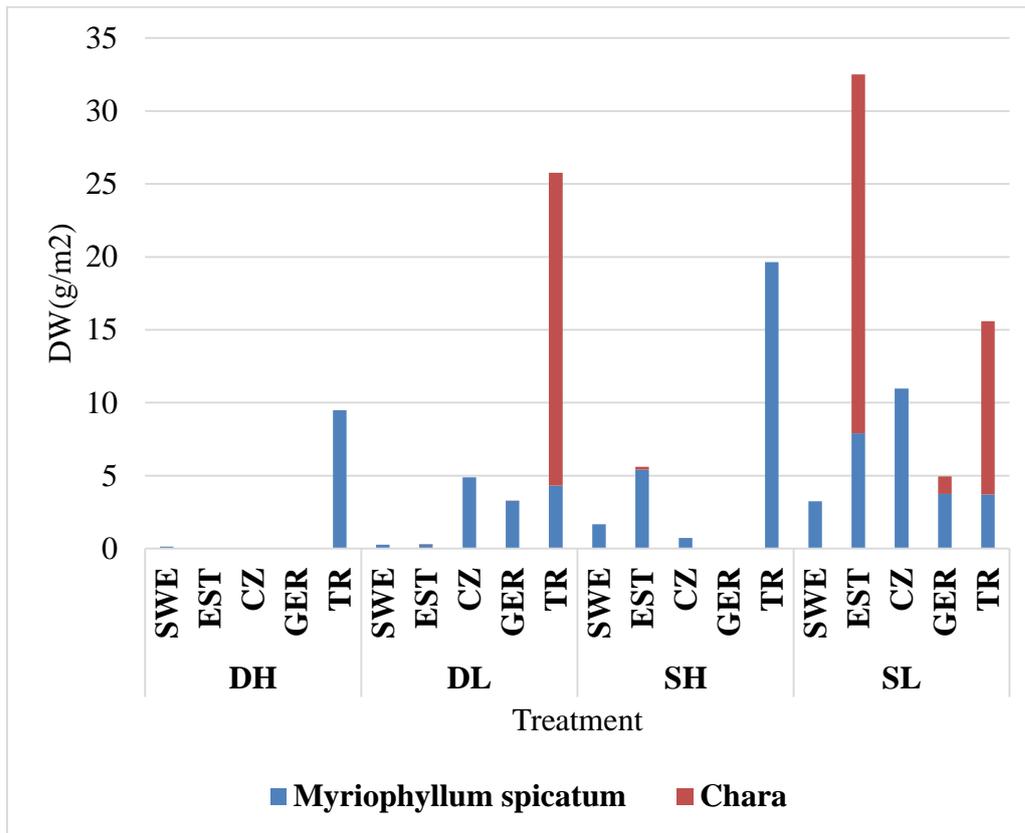


Figure 3.9 Dry weight of different macrophytes at the end of the experiment (Abbreviations of countries, SL, DH, SL and DL, explained at Fig. 3.2). Countries are ordered according to increasing temperature from left to right.

Apart from ANCOVA which only investigates the effects of fixed factors and a covariate, measured variables including, TP, TN, Chl-a, Kd, water level change and mean air temperature were used in generalized linear regression model with Gaussian distribution family to observe the effects of physico-chemical parameters on PVI and total macrophyte biomass. According to GLM results, while mean air temperature had a significant positive effect on PVI% ($p < 0.005$), Kd and water level measurements throughout the experiment were found to have significant negative influence (both, $p < 0.001$). Moreover, macrophyte biomass was also negatively related with Kd and water level measurements ($p < 0.001$, $p < 0.001$, respectively).

CHAPTER 4

DISCUSSION

Clear patterns emerged from our controlled mesocosm experiment; these include i) strong combined effects of temperature and nutrient on macrophyte growth; ii) strong effect of depth and nutrient interactions on macrophyte growth, as PVI% being higher in shallow, mainly low nutrient mesocosms; iii) negative effect of nutrients on macrophytes compensated through water level decrease especially in shallow high nutrient mesocosms in warmer countries; iv) negative effects of extreme water level reduction on the macrophyte biomass.

Expectedly, nutrient concentrations differed significantly among different nutrient and depth treatments, as we added different amount of nutrients to the deep and shallow mesocosms. Significant effect of nutrient treatment on TP and TN concentrations clearly showed that nutrient loading throughout the experiment reached to the targeted levels in low vs. high nutrient. However, differences in TP and TN concentrations between SH and DH mesocosms became greater with the temperature increase, though the same was not observed between SL and DL mesocosms, possibly due to concentrations being already very low in low nutrient treatments and buffering mechanisms of macrophytes (Scheffer et al., 1993; Van Donk & Van de Bund, 2002). In warmer countries, TN concentration tended to be higher in deep and high nutrient mesocosms, which could have resulted from decreased nutrient retention in higher temperatures (Kosten et al., 2012, Coppens et al., submitted) and increased concentration of nutrients caused by high evaporation (Özen et al., 2010).

Depth-nutrient interaction had a significant effect on Chl-*a*. High nutrient mesocosms had higher Chl-*a* mostly in deep mesocosms, which may indicate phytoplankton taking advantage of high TP and TN concentrations. Since there is a possible competition between macrophytes and phytoplankton in shallow mesocosms, this effect is clearer in deep mesocosms due to low abundance or lack of macrophytes (Lacoul & Freedman, 2006).

Although temperature change did not exactly follow the latitudinal gradient due to altitude and microclimatic differences, an overall strong temperature gradient (9 °C) was found. Many studies suggest that in warmer temperatures, macrophytes reach to the surface faster, get more access to light (Barko & Smart 1981; Rooney & Kalff, 2000) with the extension of growing seasons (Patrick et al., 2012). On the contrary, in a 2-year warming experiment of mesocosms in England, warming of 3° C found out to have insignificant effect on growth of natural macrophyte communities (McKee et al., 2002). Our results seem to contradict with the findings of McKee et al. (2002), but temperature range of this experiment (3° C increase) was lower than that of our experiment (9° C).

Significant nutrient-temperature interaction on PVI % indicated that high temperature and low nutrient levels promoted macrophyte growth, with the effect of temperature being stronger. Although negative relationship between macrophyte coverage and nutrient enrichment has been shown in various studies (Fernández-Aláez et al., 2004; González Sagrario et al., 2005), in this study dense macrophyte growth was detected in high nutrient mesocosms in Turkey, possibly because of water level reduction.

Studies in subtropical (Mazzeo et al., 2003; Havens et al., 2007), northern temperate (Nõges & Nõges, 1999; Liira et al., 2010) and Mediterranean climates (Beklioglu et al., 2006) have shown that water level reduction can trigger the expansion of aquatic vegetation growth. Our results with the significant effect of depth-nutrient interaction and higher macrophyte growth in shallow high nutrient tanks, are also

consistent with the findings of mesocosm experiments conducted by Özkan et al. (2010) and Bucak et al. (2012) in Mediterranean climate zone.

There was a clear water level change in Turkey and Greece throughout the experiment that affected macrophyte growth. In Turkey 43 cm decrease in water level had a positive impact on the growth of macrophytes in high nutrient, particularly shallow mesocosms; whereas in Greece the extremely high water level decline (83 cm, leaving 7 cm water depth) had a negative effect. High nutrient mesocosms in Turkey, which experienced significant water level decrease had relatively high macrophyte coverage. This was possibly due to the combined effect of warmer temperature and low water level, with the first inducing higher shade tolerance (Kosten et al., 2011) and the latter providing higher light availability for macrophytes (Duarte, Kalff & Peters, 1986). Hence, water level drop compensated the effect of nutrient loading on macrophyte growth in Turkey (Beklioglu & Tan, 2008; Jeppesen et al., 2015). Having low light attenuation in both shallow and deep mesocosms, Turkey had the highest light intensity at the bottom of the mesocosms which enabled higher plant growth. In Greece which has the highest temperature, comparatively lower macrophyte biomass was found than in Turkey. As the mesocosms used in our experiments were protected against wave and wind effect, low macrophyte biomass in Greece could be attributed to extreme reductions in water level, since macrophytes could not support much growth due to low volume to surface area ratio and could dry out (Coops et al., 2003; Geest et al., 2005). Likewise, in a shallow tropical lake in Brazil (Loverde-Oliveira et al., 2009) and a shallow Mediterranean lake in Sicily (Barone et al., 2010) macrophytes disappeared after a strong water level decline and the lake ecosystem was changed to turbid dominated state. It is also known that warming (Kosten et al., 2012), water level drop (Jeppesen et al., 2015) and drought conditions (Romo et al., 2013) result in more eutrophic conditions with high occurrence of cyanobacteria. Difference between high nutrient mesocosms of Turkey and Greece could be caused by higher cyanobacteria in Greece compared to Turkey (Erdoğan et al., unpublished data). Similarly, in Greek mesocosms we found turbid water conditions with high light attenuation which is highly correlated with phytoplankton Chl-*a*, significantly

reducing PVI %. Also, probably due to fish feeding at low water level, sediment suspension in the water column and high periphyton biomass (Mahdy et al., submitted) may have affected the macrophyte growth in Greece mesocosms.

Apart from the inoculated macrophyte species, different macrophyte species were also observed during and at the end of the experiment. Higher macrophyte biomass (made up of mostly charophytes) was observed in SL mesocosms of Estonia, compared to other cold countries. Charophytes were known to dominate at low nutrient concentrations (Bakker et al., 2010), which is consistent with our findings. Occurrence of different macrophyte species could be resulted from nutrient concentrations and sediment inoculation including oospores and seeds from five local lakes in each country. Occurrence of different macrophyte species could be resulted from nutrient concentrations and sediment inoculation including oospores and seeds from five local lakes in each country.

It is acknowledged that periphyton growth (Phillips et al., 1978) negatively affect macrophytes (Sand-Jensen & Borum, 1984; Weisner et al., 1997). Results of the periphyton experiment using the same experimental set-up showed that periphyton biomass (only deep mesocosms and excluding Sweden) significantly increased with temperature (Mahdy et al., 2015). However, negative interference of periphyton growth was not observed in warmer countries despite their higher biomass, in Turkey and Greece, macrophyte growth was observed in DH and DL mesocosms (Fig. 11). Our results also support findings of Ozkan et al. (2010), where macrophytes were resilient to periphyton shading due to water level decrease during summer.

CHAPTER 5

CONCLUSION

By combining SFTS approach with controlled mesocosm experiments, we aimed to investigate the effects of climate warming on macrophyte growth at two nutrient and water levels. During the experiment the high water level decline (up to 46-83 cm) in the mesocosms was observed in Greece and Turkey, where high air temperatures were detected throughout the experiment.

Depth - nutrient and temperature- nutrient interaction had a significant effect on macrophyte growth. High macrophyte growth was observed in shallow low nutrient mesocosms of warmer countries. Despite having high nutrient levels, shallow high nutrient mesocosms of Turkey had high PVI%, possibly due to water level decrease. However, extreme water level decrease was detrimental for macrophyte growth in Greece, where comparatively lower macrophyte biomass was observed.

In conclusion, our study revealed a positive effect of temperature and water level decrease together that counterbalance the negative effect of nutrient on macrophyte growth. Our results suggest that macrophytes were resilient to nutrient addition in warmer countries where water level decline together with warmer and more light availability conditions increase the likelihood of occurrence of macrophyte growth. Strong effects of temperature on macrophyte growth in warmer countries overrode the effect of nutrient induced light reduction as seen in Becares et al. (2008) and Kosten et al. (2011) as well as water level drop (Özkan et al., 2010; Bucak et al., 2012). However, extreme water level decline resulted in strong decline of the plants. Our results therefore indicate that global climate warming could favor growth of macrophytes with water level drop even under eutrophic conditions; however combined effects of eutrophication and extreme water level reductions may also

negatively affect the development of macrophytes, if longer and intense drought periods become more common.

Further studies are needed in order to understand the effects of climate warming on macrophytes, which have noteworthy roles in freshwater ecosystems. Robust experimental designs and comprehensive protocols are crucial to obtain more accurate results. Several other effects of climate change including heat waves, flooding etc. could also be tested by mesocosm experiments. However, although experimental studies with latitudinal gradient are useful to get a wide understanding of how climate influence the ecosystems, these studies should also be supported by modelling and long-term monitoring data.

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