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Correlation of absolute chlorophyll content to the photosynthesis capacity under different management regimes in two wet grassland plants *Carex canescens* and *Phalaris arundinacea*

Bachelor thesis

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Annotation

Two wetland plants, *Phalaris arundinacea* and *Carex canescens* are grown in nutrient rich- and poor, as well as water saturated soils. In a mesocosm experiment, chlorophyll, photosynthesis, biomass and stem characteristics were monitored during the growing period to obtain information about their varying response to nutrient addition.

Declaration

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

České Budějovice, 26.02.2024 .....

Jana Adali

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Abstract

Wetlands are highly fluctuating ecosystems, which are especially prone to invasions. In this thesis two wetland plants, namely *Phalaris arundinacea*, which is a classical invasive species, and *Carex canescens*, which has a highly different, slower growth strategy, are observed under different management regimes. Abiotic conditions such as nutrient addition would cause them to alter morphological traits (chlorophyll content, photosynthesis) and thereby indirectly influence their physiology (stems, biomass). These parameters are often used to indicate plant well-being and give conclusions about their growth. Performing a mesocosm experiment under water saturated conditions allowed to compare the two species during the vegetative growing stage, by measuring chlorophyll, photosynthesis, biomass and stem characteristics (stem counts, height). Analyzing these parameters confirmed the fact that these plants follow different growing patterns and utilize the added nutrients differently. *P. arundinacea* was thriving under nutrient enriched conditions and utilized nutrients most efficiently. *Carex canescens* was more effective under non-eutrophic conditions, and could possibly outcompete *P. arundinacea* under said conditions. The experimental outcomes can be used to predict wetland vegetation changes in response to anthropological nutrient inputs, and thereby contribute to the implementation of suitable management regimes to control invasions.

Contents

1 Introduction

1.1 Wetland Ecosystem

1.1.1 Definition

Wetlands are highly fluid systems and challenging to classify, hence there is no universal definition. An example for their fluidity is their varying presence of water regarding depth and duration of flooding periods (Tande & Lipkin, 2003) resulting in moisture gradients reaching from relatively dry to flooded soils (Reddy & DeLaune, 2008). As they are typically found in transitional zones between aquatic and terrestrial environments (Tande & Lipkin, 2003; Davids, 1995), they are influenced by both systems and exhibit characteristics absent in either environment. They can vary in size and shape, ranging from a few hectares to tremendous regions encompassing hundreds of square kilometers. They occur in diverse settings, from inland, coastal, rural and urban. Unlike most common ecosystem types, which have shared characteristics, wetlands exhibit high variations among different wetland types (Mitsch & Gosselink, 2015). However, certain defining characteristics persist, which all include the presence of water for a prolonged amount of time (Kanaujia & Kumar, 2014; Mitsch & Gosselink, 2015). Water is the key factor in wetlands, as it affects the composition of the soil, which determines the type and quantity of flora and fauna present (Mitsch & Gosselink, 2015; Russo, 2008). A wetland may also be defined by having at least one of the following characteristics: the presence of water tolerant vegetation (hydrophytes), water levels at or near the soil surface, creating anaerobic conditions at some time, or a substrate that is water saturated or covered by a non-soil material (Russo, 2008).

1.1.2 Functions and Ecosystem Services

Wetlands offer a variety of important resources and functions. Wetland functions are defined as the intrinsic processes that naturally take place in these biomes, while ecosystem services are the processes that benefit society (Davidson, 1995).

Wetlands have historically been essential in supporting ancient societies with food, water, cropland and transportation. Especially in less developed areas populations rely on wetlands, with cultures and subsistences connected to wetland resources and flooding cycles (Hook et al., 1988; Kanaujia & Kumar, 2014). Wetlands are effective agents of water purification, acting as a natural buffer by sheltering aquatic ecosystems from contaminants. They are sufficient in removing pollution from different origins, such as pathogens, chemicals, organic

pollutants or nutrient excess by mechanisms such as sedimentation, uptake by plants, precipitation and filtration (Russo, 2008). This is why they are often referred to as "kidneys of the landscape" (Mitsch & Gosselink, 2015). They can be used as an indicator for upland soil health, by inspecting the compounds in it (Reddy & DeLaune, 2008). Wetlands located on coasts can act as a buffer between inland areas and marine ecosystems. For instance, marshes and mangrove wetlands absorb most ocean storms without suffering high destruction themselves (Mitsch & Gosselink, 2015). This is accompanied by erosion protection of these inland areas (Russo, 2008). Several other wetland functions of prevalent importance are described further in the following paragraphs.

1. Regulation of flooding

A main function of inland wetlands is flood control, by slowing down the flow of incoming water and increasing the lag time (Russo, 2008). The correlation between flood peaks and the presence of wetlands has been verified by studies: In areas with even a low percentage of wetland cover, water flow was significantly reduced. Consequently the removal of upstream wetlands lead to increased peak stream flow during flooding. It was established that the effectiveness of wetlands in regulating downstream flooding rises with wetland area, magnitude of the flood, proximity to an upstream wetland, and the water holding capacity of the respective wetland (Mitsch & Gosselink, 2015).

2. Primary production

Many wetlands are highly productive systems, especially regarding primary production, which is driven by photosynthesis (Mitsch & Gosselink, 2015). The total amount of C fixed by photosynthesis is called gross primary production. When plant respiration is subtracted, net primary production is obtained, which is the amount of organic matter that is actually created. The counterpart to primary production is litter decomposition, which releases all elements that were fixed by the plant back into the environment, available for renewed uptake (Van der Valk, 2006). Litter breakdown is facilitated though physical factors such as precipitation and sunlight, as well as herbivores. Subsequently it is colonized by microorganisms which utilize it as an energy source (DeLaune et al., 2013). In wetlands litter decomposition often takes place under anaerobic circumstances, where hydrolyzation reactions convert complex organic molecules (lignins and other phenolic compounds) into simpler ones (alcohols, fatty acids, formate). These can then be oxidized to CO_2 by microbes (Van der Valk, 2006).

3. Nutrient cycling

Wetlands take part in global atmospheric fluxes and biogeochemical cycles, such as nutrient cycles. This involves the transfer and transformation of nutrients from one environmental compartment to another. Wetlands frequently act as nutrient sinks, where the inflow of nutrients exceeds the outflow rates. This storage function of wetlands becomes evident when considering the C cycle (Reddy & DeLaune, 2008). The content of organic C in wetland soils is notably high with occasionally more than 40 % C, compared to surrounding upland soils with 0.5 - 2 % C (Nahlik et al., 2016). The C stored in wetlands can be released to the atmosphere in the form of CO_2, e.g. by burning of peat or hydrology modifications. As long as wetlands are conserved rightfully, the stored C will remain in place indefinitely (Mitsch & Gosselink, 2015).

1.1.3 Vegetation

Wetland vegetation has to endure stresses such as fluctuating water levels, nutrient- and sediment deficiency (Mendelssohn & McKee, 1992) and temperature extremes due to shallow water levels (Mitsch & Gosselink, 2015). An especially limiting factor is O_2 in the rhizosphere (Russo, 2008), due to water saturation and microbial consumption of the remaining O_2 in the soil pores (Van der Valk, 2006), which creates reducing conditions (Davidson, 1995). This leads to wetland plants experiencing difficulties in carrying out aerobic respiration. Lack of O_2 additionally alters nutrient availability, which can result in dangerous quantities of particular elements and organic compounds accumulating (Mitsch & Gosselink, 2015). O_2 exhaustion also causes changes in intracellular pH levels, impaired cell maintenance, hormonal alterations (Armstrong et al., 1994), stomatal closure and a reduction in photosynthesis (Pessarakli, 1996). To resist root anoxia caused by flooding, hydrophytes employ complex structural, morphological and physiological tactics (Mitsch & Gosselink, 2015), which are tightly bound to reproduction and growth cycles (Armstrong et al., 1994). Some of these mechanisms are discussed further in the following paragraphs:

1. Aerenchyma

Upon submersion, herbaceous macrophytes can develop interconnected internal air spaces, called aerenchyma (Van der Valk, 2006). Aerenchyma development improves O_2 transmission from above- to belowground regions (Mitsch & Gosselink, 2015). It reduces resistance against longitudinal gas transport as well as the O_2 demand in general (Armstrong et al., 1994). The two possible mechanisms by which aerenchyma can form are cell separation during root cortex

maturation and cell disintegration (Van der Valk, 2006). While aerenchyma is not equally distributed in the plant body, its thin lateral walls allow internal gas diffusion to occur (Mitsch & Gosselink, 2015). The distribution of gases through this structure, called a gas phase continuum, is continuously occurring (Van der Valk, 2006). The gas phase continuum is located in the cortical tissue and can range from the stomata to the root cap (Armstrong et al., 1994). In wetland macrophytes, the gas space continuum is highly developed (Van der Valk, 2006). But even without considering the effects of aerenchyma, macrophytes exhibit higher aeration levels than nontolerant species. This is due to their cell structure, which is cubic instead of hexagonal and enables higher porosities within the plant body (Armstrong et al., 1994).

2. Radial oxygen loss

Excess O_2, which is not consumed by root respiration diffuses into the surrounding soil (Van der Valk, 2006), a phenomenon called radial oxygen loss. This changes the composition of soil microbes and nutrient availability by aerating the soil surrounding the roots (Colmer, 2003). Additionally, the negative effects of certain ions, such as Mn^{2+}, Fe^{2+} and Fe^{3+} are counteracted by oxidative reactions (Mitsch & Gosselink, 2015; Armstrong et al., 1994). Radial oxygen loss leads to increased soil redox potentials and lower pore water S^{2-} concentrations. Thereby flood tolerant plants contribute to the aeration of nearby non-tolerant plants (Mitsch & Gosselink, 2015).

3. Adventitious roots

Structural adaptations like adventitious root formation are triggered by hormonal changes, especially involving ethylene (Mitsch & Gosselink, 2015). These fine roots develop on the surface of the soil during flooding. Although not the most effective way in obtaining O_2, it helps reduce the uptake of toxic compounds (Armstrong et al., 1994).

4. Lower water uptake

Plants intolerant to low O_2 settings usually have restricted water uptake, even when water is available. This could be due to decreased root metabolism. The results of reduced water intake are similar to plants reactions to drought: stomatal closure, reduced CO_2 uptake, decreased transpiration and wilting. These responses are advantageous in limiting water loss and protection from cell damage (Mitsch & Gosselink, 2015).

5. Anaerobic respiration

Under low O_2 supply, roots switch to anaerobic respiration, or fermentation. As this process generates less energy than aerobic respiration, and produces toxic end products (e.g. methanol), it is not a permanent solution (Van der Valk, 2006; Armstrong et al., 1994). As ethanolic and lactic acid fermentation do not yield sufficient energy for active growth, plants fall into a state of reduced activity until conditions improve. Although fermentation eventually leads to the formation of phytotoxic intermediates and end products, there is a mechanism which counteracts the severity of the resulting toxicity: the diversification of harmful compounds. Hence, not only ethanol, but also alanine and lactic acid are commonly produced (Armstrong et al., 1994).

1.1.4 Wetland losses and challenges

Wetlands are frequently modified to serve industry, farming and urban growth. In the US, over 50% of the wetlands present before industrialization had disappeared by the 1970s (Hook et al., 1988; Brinson & Malvárez, 2002). This is a global trend: over half of all wetlands have been lost worldwide and keep on vanishing, especially in developing countries (Mitsch & Gosselink, 2015). Various causes contribute to this ongoing decline, including the perception of wetlands as wastelands, association with pathogens and hazards and a lack of financial assistance (Hook et al., 1988). The most consequential human influences to wetlands are draining, sealing of the soil surface, mining and water contamination (Mitsch & Gosselink, 2015). One type of alteration, which falls under the category of pollution, is eutrophication (nutrient excess). It is a widespread problem in industrialized countries, especially for wetlands surrounded by intense agriculture (Maurer & Zedler, 2002). Wetlands can receive nutrients and pollutants from various sources, such as surface water, ground water, precipitation and the atmosphere (Brinson & Malvárez, 2002). Factors such as the management on upstream areas have effects on nutrient inputs (Mitsch & Gosselink, 2015). Eutrophication directly effects wetland species, with agricultural runoff promoting invasive species. Additionally, farm runoff can contain Se, which has caused the death and deformity of wildlife in the past (Mitsch & Gosselink, 2015). Wetland depletion causes environmental issues which, in turn, affect the global population. Severe outcomes might involve increased flooding, species decline, extinctions, and loss in water quality (Kanaujia & Kumar, 2014). Wetland degradation is expected to proceed rapidly in the next few decades. It requires significant changes in management regulations, strategies and institutions in order to be counteracted (Mitsch & Gosselink, 2015).

1.2 *Carex canescens*

Carex canescens is a perennial from the family *Cyperaceae,* commonly called sedges. The plant grows from compact dark rhizomes forming brownish clumps at the bottom, covered in older, dead leaf material. The thin culms stand upright and can have a height of 10-50 cm. The flat leaves are partly coated by thin sheaths and have sharp blades. The pale or silver-green color of the spikes is responsible for the name of the plant (Tande & Lipkin, 2003; Hurd et al., 1998). *C. canescens* prefers acidic to neutral pH levels and grows in the temperate regions of Europe, Asia and North America. Typical habitats are swamplands, wet meadows and lake-banks. Blooming and fruiting occur from June to August (Tande & Lipkin, 2003; Hurd et al., 1998). Many sedge species occur primarily in low management ecosystems, but some species thrive better under management such as herbicide application, raking, burning or manipulation of water levels (Anderson & Davis, 2013; Schütz, 2000). They are herbaceous and bloom and seed at various times in their lifetime (Anderson & Davis, 2013; Schütz, 2000). *C. canescens* grows mostly in low-nutrient habitats. This is represented by low nutrient uptake capabilities, which results in *C. canescens* being considered as a slow growing plant. This is not a downside as its dense leaf structure results in low tissue turnover rates and therefore greater nutrient retention (Aerts & Chapin, 2000). Further, low respiration rates, water flow and loss, and specific leaf area are characteristic of this species (Colesie et al., 2020). Sedges serve as an indicator plant: their presence helps in determining wetland borders more clearly (Tande & Lipkin, 2003). Specifically they are indicators of oligo- to mesotrophic or water saturated soils, therefore the species has faced declines with the extensive use of fertilizers (Toogood, 2005). Their (non-)existence can indicate environmental- and recent changes associated with modern land use (Schütz, 2000).

1.3 *Phalaris arundinacea*

The perennial Reed Canary Grass (RCG) or *Phalaris arundinacea* is one of 15 species of the genus *Phalaris,* belonging to the family Poaceae (Apfelbaum & Sams, 1987; Barkworth et al., 2007).The plants stems reach a height of 0.5 - 2 m, with panicles of 7 - 40 cm. The stems grow from rhizomes to form dense monocultures with radial distribution (Apfelbaum & Sams, 1987). It is native to North America, but has migrated over nearly the whole world, which explains its variability in physical appearance (Apfelbaum & Sams, 1987). Although RCG is able to adapt to a wide hydrological range, it favors moist settings (Wilcox et al. 2007). It starts spreading vegetatively in early spring by the formation of dense root structures and large quantities of aboveground matter (Adams & Galatowitsch 2005). No dormancy is required;

the seeds germinate as soon as the embryo is mature. Then they grow laterally for 5-7 weeks, followed by tillering (Apfelbaum & Sams, 1987). During the early stages of vegetative growth the plant can gain rapidly in height and biomass (Stannard & Crowder, 2001; Wrobel et al., 2009). This degree of productivity is reflected in the plants high nutrient demands (Stannard & Crowder, 2001; Ehrenfeld, 2003), which is a characteristic feature of fast, competitively growing species. Their high nutrient uptake is accompanied by low nutrient retention, due to low leaf density (Aerts & Chapin, 2000). Additional features are high water transport (Colesie et al., 2020) and low tissue lifespan (Aerts & Chapin, 2000). As an invasive species RCG competes far more aggressively for resources than conservative species (May, 2007) and thereby has the capacity to repress them by advancing rapidly into their habitat (Kercher & Zedler, 2004). It decreases biodiversity by the formation of aggressive monocultures and the overshadowing of smaller species (Wisconsin Reed Canary Grass Management Working Group, 2009). This has destructive effects on wetlands and the species living in it (Price, 2019). RCG is rarely consumed as a natural food source and its dense growth limits its use for wildlife (May, 2007). It is difficult to manage (Apfelbaum & Sams, 1987), because of its adaptability to various environmental conditions and resilience to disruptions such as grazing, burning and flooding (Stannard & Crowder, 2001; Kidd & Yeakley 2015, Maurer & Zedler, 2002). Beyond its negative implications on ecosystems, RCG exhibits several positive applications. It is efficient at eliminating N and other elements from wastewater effluent and regulates NO_3^- levels in drainage water through its extensive root system. Additionally, RCG has been effective in erosion control in environments with variable water levels (Wrobel et al., 2009).

1.4 Biomass allocation

Different parts in terrestrial plants all have designated functions: leaves are the main photosynthetic organ, roots absorb water and nutrients, stems guide the water throughout the plant body and leave-veins distribute it within the plant. The biomass allocation in these structures varies within the lifetime of a plant (Poorter et al., 2012). The rate of C accumulation is defined by the difference of C fixation during the day and C losses through respiration at night (Pessarakli, 1996). According to the functional equilibrium of plant growth, plants relocate their biomass depending on the limiting factor, promoting the absorption of the factor that is least abundant. Under low nutrient conditions, biomass is allocated to the roots (Poorter et al., 2012; Aerts & Chapin, 2000). If sunlight is limiting, biomass shifts towards the shoots, as in return less nutrients and water are required. High CO_2 content leads to increased

allocation to the roots, as photosynthesis and therefore nutrient requirements rise (Poorter et al., 2012). An indirect method for assessing biomass production is the end of season harvest. As a destructive method it is not suited to assess changes in biomass over time, such as formation and mortality of stems and leaves (DeLaune et al., 2013; Pessarakli, 1996). However, the practice of repeatedly measuring stem height and density from permanent plots (allometric relationship) enables exactly that. As it is a nondestructive method, it allows repeated measurements from the same area and is more informative about growth dynamics than harvesting. The measurement of belowground biomass is especially challenging. Generally, it involves tedious manual separation of roots from soil and is often done via soil cores (DeLaune et al., 2013).

1.5 Main nutrients and their role for plant growth

Nutrients are critical for macrophyte growth, as they are absorbed and used to produce metabolic compounds such as enzymes, amino acids and proteins, and lipids (DeLaune et al., 2013). They can be classified into mineral nutrients, which encompass macro- and micronutrients and are contained in soil, while non-mineral nutrients are found dissolved in water and in gaseous form in the air (e.g. H, C, O). Macronutrients like N and P usually are limiting and required in larger quantities, compared to micronutrients such as Fe, Cu, Mo, Mn, B and Zn. Secondary nutrients like Ca, Mg or S also are classified as macronutrients, although they are needed in smaller quantities (May, 2007). In this thesis the focus is on the main nutrients N, P, and K.

K is the element most closely associated with stomatal regulation. Through the K ion pump, the turgor of guard cells is maintained. These are responsible for the opening and closing of pores in the stomata, enabling gas exchange with the atmosphere (Pessarakli, 1996). Reduced K absorption is connected with decreased stomatal conductance and closure (Hook et al., 1988). In practice, K limitation occurs rarely (Aerts & Chapin, 2000), mainly in the case of permanent flooding (Pessarakli, 1996).

P is a constituent of molecules crucial for aerobic respiration, including ATP and NADH. Additionally it makes up the physical structure of the DNA backbone. Insufficient P levels can slow down photosynthesis rates. However, P plays a more vital part in resource allocation to vegetative vs. generative plant tissues (Aerts & Chapin, 2000) in that deprivation of P mainly impacts growth parameters such as shoot DW, leaf count and leaf area (Pessarakli, 1996).

The most essential nutrient for plant development is N (Pessarakli, 1996), which can exist in various forms. The most reduced form used in plant uptake is NH_3. Naturally, N exists in more oxidized forms, like NO_3^- and NO_2^- (Aerts & Chapin, 2000; Pessarakli, 1996), which have to be converted into more available forms before plant uptake (Pessarakli, 1996). The conversion of atmospheric nitrogen (N_2) into this organic form is not facilitated by plants but by soil microorganisms in the course of N fixation. Presently, NH_3 is synthesized from N_2 for fertilizer production by the Haber-Bosch method at much higher rates than natural fixation processes. Wetlands often receive agricultural runoff, making them crucial for returning surplus N to the atmosphere via denitrification. This process requires the presence of organic C, as well as an aerobic and reducing atmosphere (Mitsch & Gosselink, 2015).

The vegetation of an ecosystem primarily absorbs the form of N it is acclimated to. Plants from high NO_3^- environments succeed in NO_3^- reduction, compared to species from other habitats (Aerts & Chapin, 2000). Although leaf tissue DW is relatively low in N (around 3 %) it plays a vital role in photosynthesis capacity and productivity (Nijs et al., 1995). This is due to high allocation of N to photosynthetic compounds, such as RuBisCO, the central enzyme in photosynthesis (Luo et al., 2021) as well as thylakoid proteins (Nijs et al., 1995). N-based proteins are crucial in photosynthesis' light-captured electron transport and C metabolism (Peterson et al., 1999).

Plant growth is not only influenced by the concentrations of individual nutrients, but also often involves co-limitation by several nutrients (Güsewell et al., 2005; Aerts & Chapin, 2000; Woo & Zedler, 2002). Species richness correlates with the ratio of N:P, which should ideally be around 15, suggesting that imbalances could be counteracted by fertilizer application. This holds true only for ratios above 20, and it mainly promotes the growth of common species rather than scarce ones (Güsewell et al., 2005). Alternative methods, such as regulation of hydrology, offer slower means to manage N and P separately: N mineralization is closely linked to water quantity, while P mineralization depends on water quality and pH levels (Braakhekke & Hooftman, 1999).

Due to their distinct biochemical cycles, N and P have varying time dependent availability in the soil. N gets deposited into the soil via the atmosphere, which leads to low initial N rates that continuously increase with soil maturation. In turn, P originates from rock erosion, leading

to abundance in early soil stages, but becoming a limiting factor with time. This indicates that soils could switch from N to P limitation. Most ecosystems are N- rather than P limited. N moves easily through the soil, leading to potential N leaching and gaseous N losses across ecosystem borders. Unlike P, the N contained in debris is less biologically available as it is C bonded, while P, with its ester bonds is readily available for plant uptake (Aerts & Chapin, 2000).

The availability of N, P and K has a significant impact on wetland plant communities, affecting their productivity and structure (Güsewell et al., 2002). Initially, nutrient enrichment promotes taller and denser growth, higher biomass and a lower root : shoot ratio (Maurer & Zedler, 2002), which favors species with rapid development and high light acquisition features. This preference for fast-growing species excludes and overshadows slow-growing species with less efficient nutrient uptake. As a result, biodiversity declines with only a few common species replacing a multitude of uncommon ones (Waterton et al., 2022). Additionally, the fast turnover rates bring about a high amount of plant litter, which accumulate and bury smaller species. The release of these nutrients into the water column subsequently promotes algal growth, by disturbing the water chemistry and causing anaerobic conditions (Sorrell, 2010).

1.6 Photosynthesis

1.6.1 Description

Photosynthesis and respiration contribute to the C balance of a plant, as well as to variations in energy fluxes (Colesie et al., 2020). 90% of the water and C exchanges between the atmosphere and biosphere are the result of photosynthesis (Wang et al., 2020). Photosynthesis yields a majority of plant DW (Colesie et al., 2020) by converting CO_2, water and light energy into C compounds and O_2 as a byproduct. A majority of the fixed C is set free into the atmosphere via respiration, releasing energy (Leegood et al., 2006). Even though plant parts such as stems, floral parts and branches can be photosynthetic, the main site of C fixation are the leaves. These contain a vast number of chloroplasts and have the optimal use of surface area. Photosynthesis is sensitive to external stresses, resulting in damage to chloroplast structure, reduced use of solar energy, alterations in pigment composition and disabled ATP production (Pessarakli, 1996). Photosynthesis is regulated by various molecules, RuBisCO being the most prevalent (Pessarakli, 1996). It serves as the first enzyme in the Krebs cycle. Due to its low efficiency, adequate concentrations are required to reach sufficient photosynthesis catalysis (Vicente et al., 2011). However research suggests that RuBisCO

effectiveness is determined by the activated amount, rather than its total amount (Pessarakli, 1996).

Naturally, photosynthesis rates continually increase in the morning, peak around midday, and then decline in the afternoon resulting in a parabola shaped curve. Midday depression is an exception. In this case, photosynthesis rates drop after the initial peak in the forenoon and experience another smaller peak in the afternoon. This phenomenon frequently occurs on days with high sunlight irradiation, due to photoinhibition, but also due to low air moisture and high air temperature (Pessarakli, 1996).

1.6.2 In vivo measurement

Measuring photosynthetic rates gasometrically offers a nondestructive way to obtain insights about plant functioning and productivity. In the field, Infrared Gas Analyzers (IRGAs) are the most common tool to measure CO_2 fluxes. Modern IRGAs are typically open systems, which means that the air inside the chamber is purged of moisture and CO_2. Subsequently, they are added to the system in known concentrations, through an attached gas reservoir with known inflow rates. This allows to determine the exact difference in gas uptake/production by the plant. Additionally, there is the need to achieve stable light intensities for measurements, either by choosing a measuring day with minimum sunlight variation (subject to individual judgement and luck) or by utilizing an artificial light source (DeLaune et al., 2013; (Pessarakli, 1996).

1.7 Chlorophyll

1.7.1 Description

Chlorophyll (Chl) is the plant pigment responsible for the characteristic green color in plants, green algae and cyanobacteria. It is abundant in the thylakoid membranes within the chloroplasts, which are situated in the leaf's mesophyll layer. The molecule consists of a Mg^{2+} ion bound to four pyrrole rings via N groups. This large, cyclic and planar molecule is called a tetrapyrrole. The molecules character and absorption spectrum are largely determined by the side chain. Chl a, for example, has a long hydrophobic tail, which connects it to other hydrophobic compounds in its environment (Pareek et al., 2017). Chl's main function is the conversion of solar energy into chemical energy in the process of photosynthesis. It absorbs solar radiation of blue and red wavelengths and reflects a green spectrum. There are various subgroups of Chl. In this thesis, focus will be on Chl a and b only. Chl a is the primary donor in the light harvesting complexes and reaction centers of both photosynthetic systems. The

absorption peak can be located at 420 nm and 660 nm in organic solvents (Pareek et al., 2017). Chl b, an accessory pigment (Pessarakli, 1996) of yellow color, is found in higher plants and green algae. It absorbs blue light and gives absorption peaks at 453 and 625 nm in vitro. In higher plants, Chl content may act as a measure of plant health (Pareek et al., 2017) by harvesting light, promoting photosynthesis (Wang et al., 2020), and thereby driving plant metabolism (Pareek et al., 2017). Any error in Chl regulation might have fatal consequences. An excess of tetrapyrrole molecules can already result in a decrease in the enzymes responsible for removing reactive oxygen species within the cell (Pessarakli, 1996). External stress factors such as irregular water and nutrient levels, have a noticeable effect on Chl levels (Cortazar et al., 2015). The molar ratio of Chl a to b can fluctuate depending on these external factors. For instance it increases with increasing sunlight exposure (Pareek et al., 2017; Kitajima & Hogan, 2003) as well as under N shortage (Kitajima & Hogan, 2003). Generally the ratio is around 3:1 in vascular plants (Pareek et al., 2017; Pessarakli, 1996).

1.7.2 In vitro measurement

Quantitative Chl determination requires choices like selection of solvent and spectrophotometer, with these parameters affecting the absorption maxima; polar solvents, for example, raise the absorption maxima. When measuring the absorption maximum, it is vital to use the appropriate equation for calculating concentrations (Lichtenthaler & Buschmann, 2001). This requires using coefficients specific to each pigment at a specific solvent and wavelength (Pessarakli, 1996). The Lichtenthaler & Buschmann (2001) protocol describes a destructive method for the measurement of Chl and carotenoids using UV-VIS Spectroscopy. Destructive methods are suited when the sample tissue will not undergo further analyses (Hardwick et al. 1973). One limitation of this method is that Chl extracts cannot be stored for extended time periods without pigment concentrations decreasing due to the light sensitivity of the molecule (Lichtenthaler, 2001).

2 Aims and Hypotheses

The objective of this study was to determine the early season effect of nutrient addition on the physiological (Chl content, photosynthesis) and morphological (stem number, height and biomass allocation pattern) features in two wet grassland species, *C. canescens* and *P. arundinacea*. The following hypotheses were tested in this study:

1. Fertilization will have a positive effect on physiological and morphological parameters in both plant species.
2. Fertilization favors *P. arundinacea* and will increase its growth to a greater extent than that of *C. canescens.*
3. *P. arundinacea*, which is expected to have higher photosynthetic rates than *C. canescens*, will also have higher Chl contents.
4. Photosynthesis rates are expected to correlate closely with Chl contents in both species.

3 Materials and Methods

3.1 Experimental setup

The experiment was carried out at the University of South Bohemia. *C. canescens* and *P. arundinacea* plants were grown from seeds in 2020. Therefore, two-year old plants were planted on the 21st of April 2021 into pots filled with a 2:1 mixture of sand and peat, with each pot measuring 15*15*20 cm. Pots were put into basins and water levels were adjusted to achieve water saturation in soil (7 cm below soil surface). A total number of 144 plants were equally distributed to 12 basins. In May, half of the basins of each plant group were selected randomly to achieve fertilization (350 kg ha^{-1} with 15 % of N, P and K, LOVOFERT NPK 15-15-15 (Lovochemie, CR). These basins were fertilized three times in the course of the growing season. After potting the plants were given one month to adjust to the new environment.

3.2 Plant measurements

Plant height was measured and the stem number of all plants counted at 2-3 week intervals starting in May 2021. These measurements were taken on the following dates: May 20, June 4 and June 16, 2023. Selected plants were harvested for biomass in early July. The plants were removed from the pots, and the roots carefully washed to remove adhering soil. Then the plants were sectioned into aboveground and belowground parts. The samples were then dried and weighed to obtain the dry weight [g DW]. Total DW (DW_{total}), as well as aboveground

(DW_{above}) and belowground DW (DW_{below}) and the root-shoot ratio (R:S) were determined. The plants which were harvested for biomass were not used for any subsequent measurements. Then two-tailed, paired two sample t-test for means were computed to compare for significant differences between treatments and months.

3.3 Photosynthesis

Photosynthesis was measured in vivo right before each round of counting. For this, light response curves with different levels of photosynthetically active radiation (PAR = 0, 25, 50, 100, 200, 400, 800, 1000, 1200, & 1500 µmol m^{-2} s^{-1}) were determined on randomly selected plants, each representing one category of *P. arundinacea* vs. *C. canescens* and fertilized vs. nonfertilized. The number of measured replicates per category varied for each month, depending on the weather conditions, with a minimum of three for May, nine for June and two for July. For this a portable infra-red gas analyzer (IRGA: Li-COR 6400A Portable Photosynthesis System, Lincoln, Nebraska USA) was used. For the measurements, healthy leaves of a similar ontogenetic stage (second or third leaf from the top) were placed in the measurement chamber. Measurements were taken from 9 am to 4 pm to guarantee sufficient irradiance. Light response curves were generated using the Solver function in Excel as described by Brown (2001). The formula for fitting the curves was obtained from Lobo et al., (2013; equation 6 for a nonrectangular hyperbola-based model). The fitted, or corrected photosynthesis values are referred to as estimated photosynthesis (EPS; [µmol m^{-2} s^{-1}]). The dependence of EPS on the photosynthetically active radiation PAR [µmol m^{-2} s^{-1}] was captured by creating light response curves. In addition, these curves were used to determine the maximum photosynthesis rate P_{max} [µmol m^{-2} s^{-1}], quantum yield QY [m^{-2} s^{-1}], light compensation point LCP [µmol m^{-2} s^{-1}] and dark respiration R_d [µmol m^{-2} s^{-1}] for each measured plant. Average values and standard deviations were calculated for each group. Subsequently, single factor ANOVAs were computed in Excel to determine significant differences between species, treatments and months.

3.4 Chlorophyll

The respective leaves used in the photosynthesis measurements, were cut near to the chamber and stored in plastic bags protected from light. Leaf areas were scanned using the WinRHIZO image analyzing software (Regent Instruments Inc., Canada). Subsequently the samples were stored in a cool room (4°C) for approximately 24 hours, weighed and freeze dried. Again, weights were taken and the water-deprived samples were pulverized using a mill for small

sample sizes (Laboratory Mixer Mill MM 400, Retsch, Düsseldorf, Germany). The milling was carried out at a frequency of 28.0 Hz for 3-3.5 min until samples were fully ground. The resulting sample powder was used for Chl determination by UV-VIS spectroscopy. The required quantities of powder were weighed (Mettler Toledo microscale) and then extracted in 80 % acetone for 3 min at concentrations within the detection range of the spectrophotometer (*P. arundinacea*: 3 mg mL^{-1}, *C. canescens*: 1.5 mg mL^{-1}). Subsequently, the samples were centrifuged (3K30 Centrifuge, Sigma Laboratory, Osterode am Harz, Germany) with a relative centrifugal force of 20 000 and a speed of 13 375 for 4 min. 1 mL of the supernatant was pipetted into a glass semi microcuvette and read in a spectrophotometer (Specord 210 PLUS, Analytik Jena GmbH, Germany) in a range of 380 to 750 nm. Pigment concentrations were calculated using equations 1-3 from Lichtenthaler, 2001:

(1) Chl a ($\mu g\ mL^{-1}$) = 12.25 ($A_{663,2} - A_{750}$) - 2.79 ($A_{646,8} - A_{750}$)

(2) Chl b ($\mu g\ mL^{-1}$) = 21.50 ($A_{646,8} - A_{750}$) - 5.10 ($A_{663,2} - A_{750}$)

(3) Carotenoids ($\mu g\ mL^{-1}$) = (1000 ($A_{470} - A_{750}$) - 1.82 c_a - 85.02 c_b) / 198

Special attention was paid to the turbidity of the extract, as solid plant particles lead to a higher absorbance and thereby falsify the results. That is why the calculated concentrations from equation 1-3 were background corrected (Connan, 2015) by subtracting the respective absorption at 750 nm. Now it was possible to calculate the concentration of Chl a and b per leaf area [$\mu g\ cm^{-2}$] as well as per leaf DW [$\mu g\ mg^{-1}$]. Using the photosynthesis data from section 3.3, Chl was related directly to photosynthesis. Additionally, the Chl a to b ratio (Chl a:b) was computed and significant differences were tested in Excel with two-tailed, paired two sample t-tests for means.

3.5 Regression EPS vs. Chl

Regression curves were created, illustrating the dependence of EPS to total Chl content (a+b) on area and DW bases, resulting in one curve per plant and treatment group. For these curves, a PAR value of 800 was chosen, as it represented the ambient light conditions on the measuring days. Higher PAR values yielded comparable results, therefore a medium PAR value, with guaranteed light saturation was chosen. Determination of light saturation is possible by studying the light response curves to each plant graphically: the graph reaches a plateau, indicating light saturation. To assess the correlations across treatments and months, the slopes of the various regression lines were compared with t-tests using the Real Statistics Resource

Pack in Excel. Subsequently, a cluster analysis was done using K-means clustering (Statistics Kingdom, Melbourne, Australia).

4 Results

4.1 Plant measurements

Insights about the biomass production of *C. canescens* and *P. arundinacea*, for both treatment classes respectively, are given in Figure 1.

Fertilized *P. arundinacea* had the highest DW (DW_{above}: 1.75 ± 1.46, DW_{below}: 1.62 ± 0.92, DW_{total}: 3.37 ± 2.08), while nonfertilized *P. arundinacea* was comparable to fertilized *C. canescens* ($p > 0.05$). DW was the lowest in unfertilized *C. canescens* (DW_{above}: 0.38 ± 0.17, DW_{below}: 0.52 ± 0.18, DW_{total}: 0.95 ± 0.40). Fertilization increased the mean DW_{above}, DW_{below} and DW_{total} in both species, being especially greater in *C. canescens* ($p < 0.001$ for DW_{above} and DW_{total}, and $p < 0.01$ for DW_{below}). However, *P. arundinacea*, showed no significant change in DW_{above} ($p > 0.05$), but significantly changed in DW_{below} and DW_{total} ($p < 0.05$). Fertilization had no significant effect on *P. arundinacea* R:S ($p > 0.05$), which stayed constant, but it significantly decreased the R:S in fertilized *C. canescens* ($p < 0.01$).

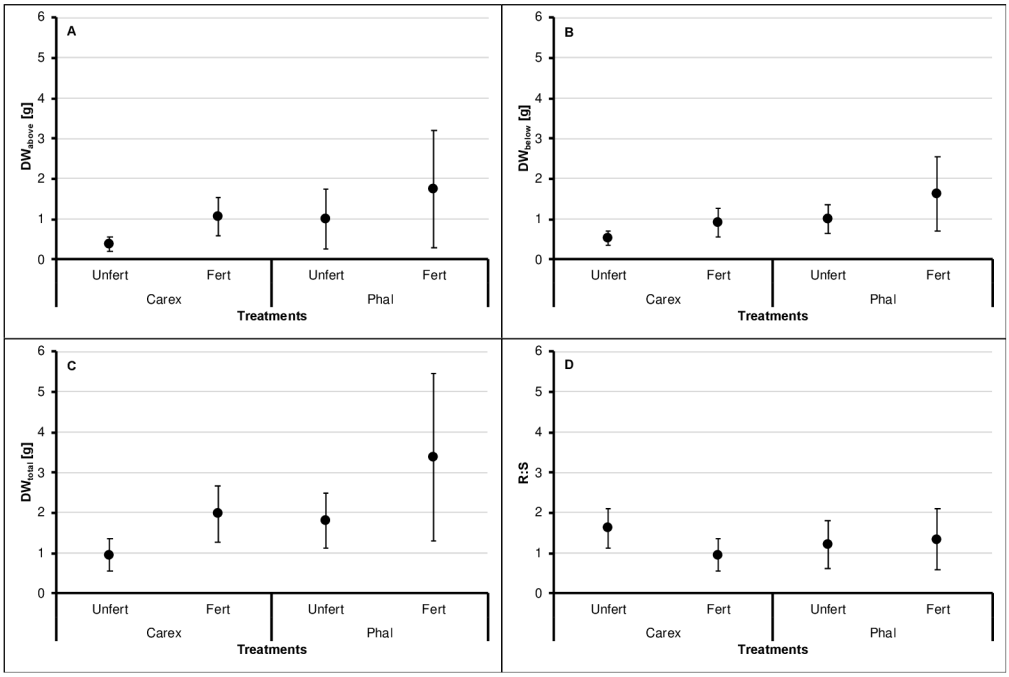

*Figure 1: Means ± SD of aboveground (DW_{above} A), belowground (DW_{below} B) and total dry weights (DW_{total} C), as well as the root-to-shoot ratio (R:S D) in Carex canescens (Carex) and Phalaris arundinacea (Phal) for all treatment types in July. Fertilization treatments: unfertilized (Unfert); fertilized (Fert = 350 kg * ha^{-1} * yr^{-1} NPK).*

The number of *P. arundinacea* stems were roughly the same (on average 8.48 ± 0.73 stems; $p > 0.05$) throughout the measurements (Figure 2). There is a slight, but non-significant increase in stem numbers in fertilized *P. arundinacea* at the end of the experiment ($p > 0.05$). Fertilized *C. canescens* started with a significantly higher stem number (14.71 ± 3.36 stems) than the unfertilized treatment ($p < 0.05$). In both treatments, *C. canescens* stem number increased significantly over time ($p < 0.001$ between weeks). Additionally, fertilized *C. canescens* stem numbers were always significantly greater compared to the unfertilized treatment for all counting events ($p < 0.01$ in May and $p < 0.001$ in June and July). *C. canescens* had significantly higher stem numbers than *P. arundinacea* throughout the summer ($p < 0.001$), especially in the fertilized treatment. Fertilized *C. canescens* in July had the highest stem number of all groups (26.75 ± 5.66 stems). The difference between *C. canescens* and *P. arundinacea* increased significantly throughout the experiment ($p < 0.001$).

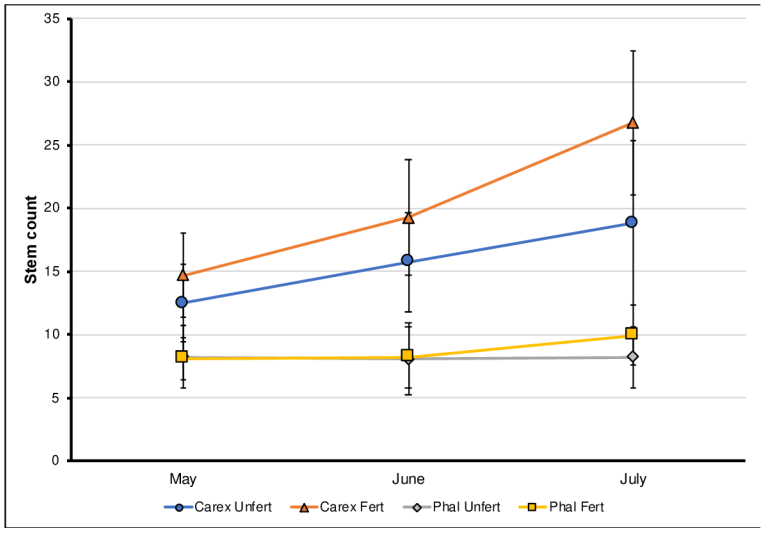

*Figure 2: Means ± SD of total stem counts of Carex canescens (Carex) and Phalaris arundinacea (Phal) for all treatment types in the three sampling events (May, June, July). Fertilization treatments: unfertilized (Unfert); fertilized (Fert = 350 kg * ha^{-1} * yr^{-1} NPK).*

Fertilized *C. canescens* had a significantly greater number of new stems in May compared to the other treatments ($p < 0.01$), which did not differ from each other (Figure 3). All groups produced significantly fewer stems in June ($p < 0.001$) with the unfertilized *P. arundinacea* having less stems overall than in May (Figures 2 & 3). This situation was reversed by July with especially the fertilized treatments producing significantly more stems compared to June ($p < 0.001$), while new stem formation remained similar in the unfertilized treatments ($p > 0.05$).

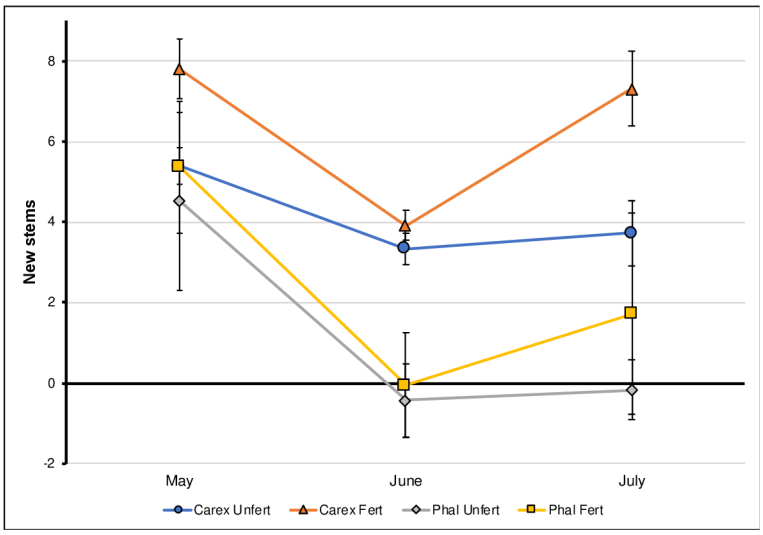

*Figure 3: The count of new stems (means ± SD) for Carex canescens (Carex) and Phalaris arundinacea (Phal) for all treatment types in the three sampling events (May, June, July). Fertilization treatments: unfertilized (Unfert); fertilized (Fert = 350 kg * ha^{-1} * yr^{-1} NPK).*

The respective heights for all groups are portrayed in Figure 4. Initial *P. arundinacea* stem heights were significantly higher (38.62 ± 9.13 cm) than those of *C. canescens* (29.21 ± 3.79 cm; $p < 0.001$). The stem height for both *P. arundinacea* treatments was highly similar in May and continuously increased for both treatments over the course of the experiment ($p < 0.001$ between measurements), attaining 61.72 ± 13.19 cm in July. Fertilization had no significant effect on the height of *P. arundinacea* ($p > 0.05$). *C. canescens* stem heights were constant, being similar between all three measurements ($p > 0.05$).

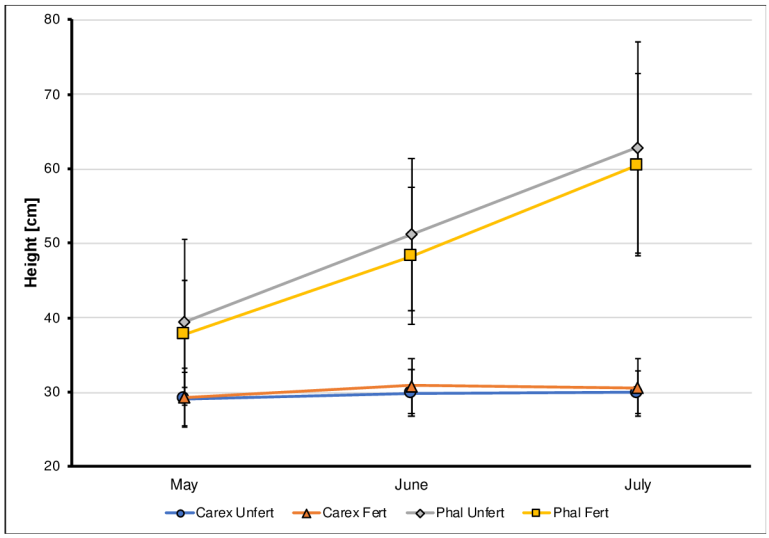

*Figure 4: The influence of treatment- and plant group on height (means ± SD) for Carex canescens (Carex) and Phalaris arundinacea (Phal) for all treatment types in the three sampling events (May, June, July). Fertilization treatments: unfertilized (Unfert);); fertilized (Fert = 350 kg * ha^{-1} * yr^{-1} NPK).*

4.2 Photosynthesis

The photosynthesis curve in Figure 5 illustrates the P_{max} for all treatment groups. For *C. canescens*, there was a significant difference in P_{max} between the unfertilized and fertilized treatments ($p < 0.05$). The unfertilized *C. canescens* had a mean P_{max} of 3.99 ± 1.26 in May, while the fertilized had a significantly lower P_{max} of 1.29 ± 0.94 ($p < 0.05$). The fertilized *C. canescens* group had a substantial rise in P_{max} in the following months, reaching 9.15 ± 2.00 by June, which was significantly greater than the unfertilized group ($p < 0.05$) that month. From June to July, no drastic changes in P_{max} were observed for both *C. canescens* treatments. In May, fertilized *P. arundinacea* had a slightly lower P_{max} (8.40 ± 7.55) than the unfertilized group (9.34 ± 3.32), but this difference was not significant ($p > 0.05$). This behavior changed by June, when P_{max} for the fertilized *P. arundinacea* increased to 10.01 ± 1.79 ($p > 0.05$), whereas the unfertilized group experienced a significant fall to 4.57 ± 2.14 ($p < 0.05$). The fertilized *P. arundinacea* reached a maximum P_{max} in July with 11.01 ± 7.06 which was greater than for the unfertilized group in July.

In general, P_{max} in the fertilized groups was lower than in unfertilized groups in May, but increased significantly over the following months, exceeding their unfertilized counterparts ($p < 0.01$ for *P. arundinacea*; $p < 0.05$ for *C. canescens*).

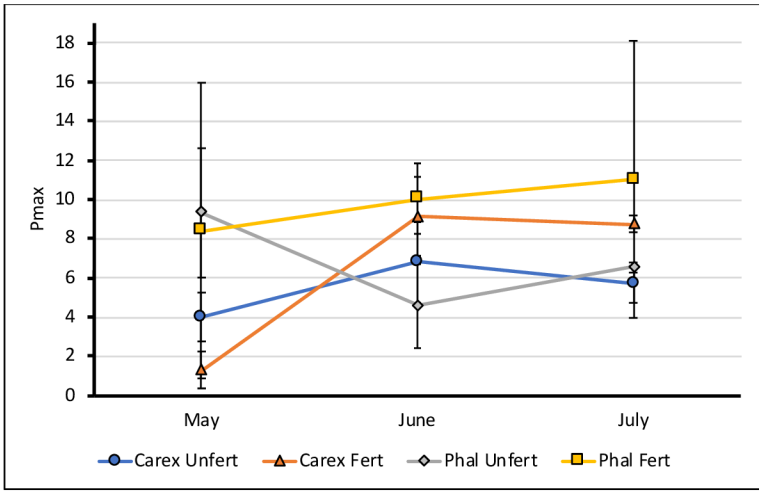

*Figure 5: Pmax curves [µmol CO_2 m^{-2} s^{-1}] at a light intensity of 800 PAR [µmol m^{-2} s^{-1}] for Carex canescens (Carex) and Phalaris arundinacea (Phal) for all treatment types in the three sampling events (May, June, July). Fertilization treatments: unfertilized (Unfert); fertilized (Fert = 350 kg * ha^{-1} * yr^{-1} NPK).*

Tables of additional photosynthetic parameters such as LCP, R_d and QY are enclosed in the appendix for each month (Appendix 1-3).

Both *C. canescens* groups had similar, high LCPs in May ($p > 0.05$; Appendix 1). That month the unfertilized group had the highest mean LCP of all groups, with 46.70 ± 14.40. LCP then

decreased by June with *C. canescens* having a significant decline ($p < 0.001$ for unfertilized *C. canescens*; $p < 0.05$ for fertilized *C. canescens*). Both groups had highly similar values in June (on average 13.53 ± 4.59). Unfertilized *C. canescens* decreased further in July, while the fertilized group increased until then, having the highest LCP of all groups in July. Regarding *P. arundinacea*, both groups had a similar LCP in May (20.06 ± 8.95 for the fertilized group, with the unfertilized group only slightly lower, $p > 0.05$). Their LCP decreased from May to July, with the fertilized *P. arundinacea* being significantly lower than at the start of the experiment ($p < 0.05$). The overall trend for all treatment's LCP is a decrease over the growing season. The general SD was notably high, especially in May, being approximately 30-60 % of the means. In May, unfertilized *C. canescens* had a mean Rd of 1.51 ± 0.55, while the fertilized group had a slightly lower mean R_d of 0.96 ± 0.47 ($p > 0.05$; Appendix 2). In June, both groups experienced a decrease in R_d, with the unfertilized group falling to 0.64 ± 0.30 ($p < 0.01$) and the fertilized group to 0.75 ± 0.10 ($p > 0.05$). This decrease continued until July without any recovery. The *P. arundinacea* groups had comparable R_d values to *C. canescens* in May ($p > 0.05$), with unfertilized *P. arundinacea* having a mean R_d of 1.28 ± 0.47 while fertilized *P. arundinacea* exhibited an insignificantly lower mean R_d of 1.14 ± 0.28 ($p > 0.05$). In June, R_d dropped for both groups, with the unfertilized group sinking to 0.75 ± 0.23 ($p < 0.05$) and the fertilized to 0.68 ± 0.22 ($p < 0.05$). As in the *C. canescens* groups, the decrease in R_d towards July continued. Over the course of the growing season *C. canescens* retained a consistent QY, as depicted in Appendix 3. It started at 0.04 ± 0.03 in May for both treatments and did not change significantly afterwards ($p > 0.05$). *P. arundinacea* exhibited a slightly different pattern. Both the fertilized and unfertilized plants had a QY of 0.07 ± 0.03 in May. QY did not vary significantly after that for either the unfertilized or fertilized *P. arundinacea* ($p > 0.05$).

4.3 Chlorophyll

Ratios of Chl a:b, which were computed for all treatment groups and months are shown in Table 1. The results were identical when computed per leaf area or DW.

In May all groups showed no significant difference from each other, with the average ratio value of 2.86 ($p > 0.05$). By June all groups exhibited increased ratios, with *P. arundinacea* having the highest (4.37 ± 0.68, $p > 0.05$ for the fertilized treatment and 4.01 ± 0.53 ($p < 0.05$) for the unfertilized treatment. Both *C. canescens* groups differed significantly from this peak value ($p < 0.01$ for unfertilized *C. canescens* and $p < 0.001$ for fertilized *C. canescens*). Fertilized *C. canescens* had the lowest average value (2.98 ± 0.25). For July there were only

two samples per category, therefore no t-tests were conducted for that month. All plant groups decreased their a:b ratio from June to July. Just fertilized *C. canescens*, which had the lowest ratio in June increased slightly until July.

Table 1: Ratios of average Chl a:b content are shown for all treatment combinations throughout the growing season. The values are identical when computed on an area [µg cm^{-2}] vs. mass [µg mg^{-1} DW] basis.

	May	June	July
Carex Unfert	2.92 ± 0.09	3.43 ± 0.43	2.99 ± 0.08
Carex Fert	2.83 ± 0.14	2.98 ± 0.25	3.17 ± 0.03
Phal Unfert	2.79 ± 0.10	4.01 ± 0.53	3.07 ± 0.18
Phal Fert	2.90 ± 0.08	4.37 ± 0.68	2.95 ± 0.17

4.4 Regression EPS vs. Chl

The following graph (Figure 6) shows the relationship between EPS at (PAR = 800) and Chl (a+b) content per leaf area, as well as per dry weight (DW). This gives insights about the dependence of photosynthesis capacity on Chl content in leaves, and how these parameters can be influenced by fertilization.

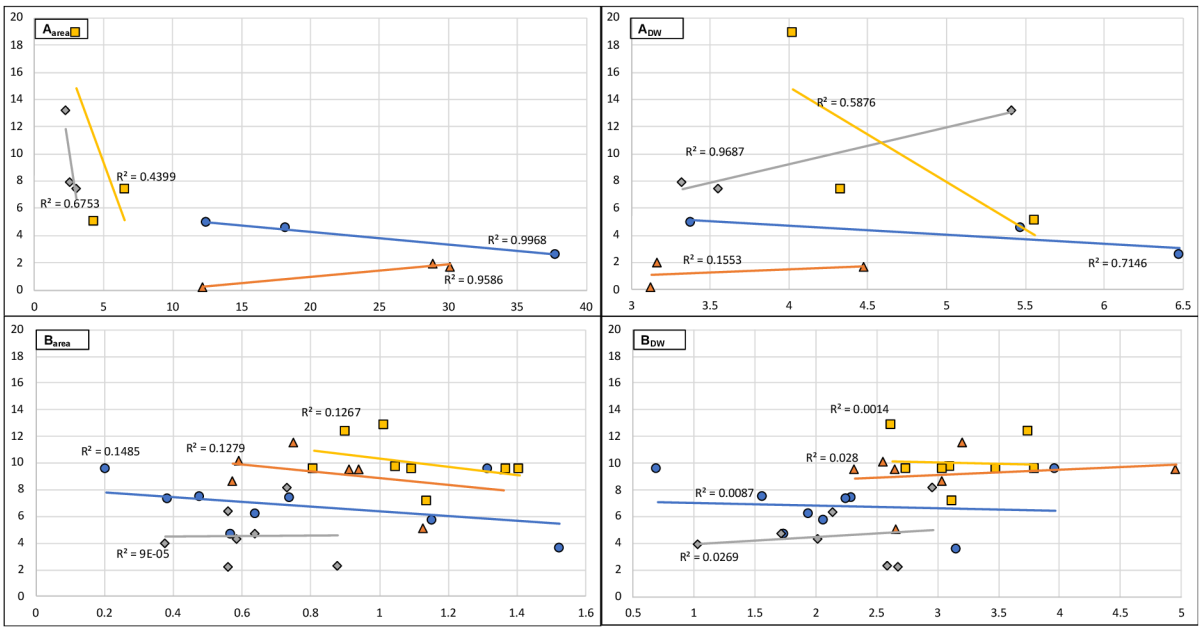

Figure 6: Dependence of corrected Photosynthesis EPS [µmol m^{-2} s^{-1}] on total Chl (a+b) for May (A) and June (B) for all treatment groups (● C.canescens unfertilized, ▲ C.canescens fertilized ◆ P.arundinacea unfertilized, ■ P.arundinacea fertilized). The values are computed on an area basis [µg cm^{-2}] for the graphs on the left, as well as on a DW basis [µg mg^{-1} DW] for the graphs on the right. For the July dataset no regressions were computed, as there were not enough datapoints.

Considering the regression graphs on an area basis, in May the unfertilized *C. canescens* had the highest range in Chl content (12.49 - 37.80) compared to the *P. arundinacea* groups ($p < 0.05$). These Chl contents did not reflect the EPS, which only ranged from 2.56 to 4.91. Only

fertilized *C. canescens* had lower EPS (0.20 - 1.96). It displayed a Chl content of 12.17 - 30.13. The EPS : C ratio was lowest in this group, indicating low photosynthetic efficiency. The *P. arundinacea* groups on the other hand had comparable and low Chl concentrations (2.24 - 3.02 for the unfertilized group and 3.01 - 6.55 for the fertilized treatment, $p > 0.05$). Nonfertilized *P. arundinacea*s EPS ranged from 7.47 - 13.17, making it the most efficient in terms of EPS : C ratio. The fertilized group was slightly less efficient and had an EPS span of 5.04 - 18.85. The slopes of the regression curves showed no statistical significance, except for *C. canescens* fertilized and unfertilized ($p < 0.05$). K-means cluster analysis showed clustering according to species (Appendix 4).

From May to June none of the regression curve slopes changed significantly ($p > 0.05$).
The Chl contents in both *C. canescens* groups sunk significantly from May to June ($p < 0.05$ for nonfertilized *C. canescens* and $p < 0.01$ for fertilized *C. canescens*). For *P. arundinacea* they stayed in a comparable region ($p > 0.05$). In June unfertilized *P. arundinacea* displayed the lowest Chl (0.38 - 0.88) and EPS (2.21 - 8.19) of all groups. Its fertilized counterpart had the second highest photosynthetic efficiency with Chl contents of 0.81 - 1.41 and an EPS of 7.11 - 12.79. Only fertilized *C. canescens* was more efficient, with relatively high Chl (0.57 - 1.36) and EPS (5.07 - 11.52) outputs. Unfertilized *C. canescens* had the broadest span in Chl contents, from 0.20 to 1.52, while also having relatively low EPS (3.54 - 9.54), making it the third efficient group. There was no significant difference between any of the regression slopes in June ($p > 0.05$). The clustering occurred according to treatment that month, as shown in Appendix 5. There were never any significant differences between any groups regression slopes between the months May and June.

On a DW basis it is apparent that the span width in Chl content became less than on an area basis ($p < 0.05$ for all groups except fertilized *P. arundinacea*), while EPS did not change. Even though the EPS:C ratios changed, the order of photosynthetic efficiencies stayed the same. While nonfertilized *C. canescens* had extraordinarily high Chl contents on an area basis, they were almost 80 % lower (3.38 - 6.48) on a DW basis ($p < 0.05$). With the EPS staying constant, unfertilized *C. canescens* is now the third efficient group when it comes to the EPS:C ratio. Fertilized *C. canescens* is even less efficient, with still low EPS values and Chl contents of 3.13 - 4.48, which also significantly differ from the values when computed per area ($p < 0.01$). Same as on an area basis, nonfertilized *P. arundinacea* is the most efficient group on a DW basis. It's Chl content is approximately half (3.32 - 5.42) on a mass basis, as opposed to

area ($p > 0.05$). For the fertilized *P. arundinacea* group the area vs. DW Chl values did not differ significantly from each other as well ($p > 0.05$). This group was the second most efficient after its nonfertilized counterpart.

The DW regression graphs for June look similar to their counterpart per area. Again, nonfertilized *P. arundinacea* is the least efficient group, with a Chl content of 1.03 - 2.96. Fertilized *C. canescens* is slightly more effective, with a Chl content of 2.32 - 4.96, and relatively high EPS values of 5.07 - 11.52. Its unfertilized counterpart decreased its Chl content from May to June to 0.70 - 3.97, while its EPS increased approximately double, making it the second efficient group after fertilized *P. arundinacea*. The high EPS for *P. arundinacea* stayed approximately constant from May to June, while its Chl content decreased further. There was no significant differences between any groups, except for unfertilized *C. canescens* and *P. arundinacea* in May ($p < 0.05$). Further, no significant differences between any plant groups of May and June were observed. Again, the clustering changed from clustering according to species to fertilization, same as on an area basis (Appendix 6-7).

5 Discussion

In this experiment, the effect of fertilization was examined on two species frequently found in wetlands, *C. canescens* and *P. arundinacea*.

Fertilization increased the biomass of both species (Figure 1), thereby supporting hypothesis 1. Additionally, hypothesis 2 holds true, as *P. arundinacea* thrives most under nutrient addition and is favored by this treatment. It had the highest above-, below-, and total biomass of all groups observed. Under non-favorable conditions (nutrient absence) its biomass was comparable to fertilized *C. canescens*. This was also found in Aerts et al. (1992) and Matzek (2012), who stated that nutrient addition leads to higher relative growth rates in invasive species, as opposed to non-invasives, however without nutrient addition the relative growth rates where even comparable between the experimental groups (with invasive species only slightly performing better). Generally, the hypothesis that a competitive species is likely to exceed the biomass of a conservative species, regardless the treatment, was mostly confirmed (Wetzel & Van der Valk, 1998).

The R:S shows the different biomass allocation patterns between the groups. Generally it is known that under nutrient absence plants will shift their resources to belowground regions to maximize nutrient uptake. Under nutrient excess a shift to aboveground regions occurs, in order to maximize photosynthesis (Holaday et al., 2015; Martina & von Ende, 2013). This morphological plasticity increases survival under varying resource availabilities (Aerts, 1999), which would prove especially advantageous in fluctuating ecosystems like wetlands (Mitsch & Gosselink, 2000). Even though the high innate plasticity of *P. arundinacea* gives reason to expect a shift in R:S under nutrient absence, this was not found in our experiment: here R:S stayed constant. In *C. canescens* however, R:S decreased upon nutrient addition, indicating that more resources are being allocated to the shoots, which would serve a competitive advantage (Poorter et al., 2012; Saggar et al., 1997). In this case, the increased availability of nutrients allows the plant to focus on performing photosynthesis, instead of focusing on nutrition. Thus, our results only partially agree with the literature. However, some sources like Aerts et al. (1992) suggest that R:S is a rather rough measure for examining allocation patterns: Even though in our experiment the aboveground biomass seemed to correlate closely with P_{max}, the assumption that photosynthesis is directly linked to aboveground biomass is inaccurate, similar to comparing nutrient uptake with belowground biomass. Photosynthesis rather depends on the leaf area, while nutrient absorption depends

on the length of the roots (Aerts et al., 1991). Examining the specific leaf area and specific root length instead could prove more accurate in future experiments. The fact that *P. arundinacea* performed well regarding P_{max} gives reason to assume that (even though it did not notably change R:S), it might have shifted its SLA to maximize P_{max} in times of nutrient absence. This effect was also discussed in Aerts et al., 1991: Phenotypic plasticity allows to compensate low aboveground biomass with high SLA.

However, such a clear nutrient effect was not seen with the morphological traits. Stem count data gives first implications about a species growth patterns. The number of *P. arundinacea* stems stayed constant and low during the summer, while *C. canescens* started with a higher stem count in May, which continuously increased from May to July (Figure 2). Plant heights (Figure 4) on the other hand were thoroughly low for *C. canescens* but high for *P. arundinacea*. This gives the implication that *C. canescens* allocates its biomass horizontally and *P. arundinacea* vertically. These allocation patterns are inherent features of the observed species. *P. arundinacea* is considered a fast growing, competitive species, which responds rapidly to nutrient addition and allocates biomass into the acquisition of light (Woo & Zedler, 2002), thereby overshadowing less efficient, smaller plants (Wetzel & Van der Valk, 1998). Under adequate nutrient supply it tends to dominate the vegetation and decrease biodiversity, due to its rapid growth (Lavergne & Molofsky, 2004). *C. canescens* on the other hand is a conservative, or slow growing species (Schütz & Milberg, 1997), which at first seems to be at a disadvantage compared to *P. arundinacea*. This was not found in our experiment, when considering the morphological responses of the species to nutrient inputs. Nutrient addition did not have an effect on the plants general growth form, which is opposite of what was predicted (hypothesis 1). Our results also differ from those of Maurer and Zedler (2002) and Steckel et al. (2003), who noted a significant nutrient effect on *P. arundinacea* height and tiller production). On the other hand, Woo and Zedler (2002) found that the native, slow species did not respond to nutrient addition, which is in accordance with this thesis. This aligns logically, as *C. canescens* is well adapted to nutrient depleted soils (Schütz & Milberg, 1997) and thereby has no incentive to increase its nutrient uptake. Secondly, it is not evolutionary equipped to take up high amounts of nutrients, nor to utilize them for its metabolism. In a previous experiment, Holaday et al. (2015) found that *C. stricta* utilized less of the supplied nutrients for photosynthesis than *P. arundinacea* did, due to inherent factors such as its low photosynthetic nitrogen use efficiency and low electron transport capacity.

As predicted by hypothesis 2, fertilization clearly favors *P. arundinacea*, which consistently had the highest photosynthetic rates throughout the summer. At the beginning of the growing season, *P. arundinacea* was already more photosynthetically productive than *C. canescens*, which is also represented in the regression (Figure 6). This is in accordance with literature, which found that under eutrophic conditions, invasive species prioritize the formation of photosynthetic organs (Matzek, 2012), which allows them to perform high photosynthesis rates right away. This gives them a head start compared to slower species (Leishman et. al, 2007), which first invest more resources in a complex root system, then assimilate the compounds of the photosynthetic apparatus and only then gradually start with photosynthesis (Matzek, 2012). This also explains the high Chl values in *C. canescens:* possibly, photosynthetic compounds were already assimilated, but not utilized yet, as the focus was still on building a sound plant body. Although *P. arundinacea* is able to be productive in the early growing season (Ehrenfeld, 2003), this head start might only be temporary, as initial productivity has been connected with shorter lifespans (Matzek, 2012). It has been found that in the long term or under nutrient deficient conditions, fast growth is not the most favorable approach, nor a measure for plant well-being (Matzek, 2012; Kaštovská et al., 2015). According to Perry et al., 2004, *P. arundinacea* faces difficulties when adjusting to nutrient poor conditions. Its high photosynthesis rates are dependent on leaf N concentration and will drop if these high nutrient intakes are not maintained. Therefore species exhibiting fast traits do not tolerate fluctuations in resources well (Colesie et al., 2020). Zhang et al. (2020) found that the initial competitive advantage of fast growing species decreased throughout the growing season, and was even negative in the end. This can be seen to some extent in our experiment: when observing the P_{max} for *P. arundinacea* (Figure 5), this initial spurt in productivity was not sustained for long: nonfertilized *P. arundinacea* rapidly decreased its P_{max} and EPS, starting in June, where this costly strategy could not be maintained anymore. Holaday et al. (2015) also found a decrease in *P. arundinaceas* metabolism when subjected to low nutrient conditions. When considering the P_{max} and regressions for June it becomes evident that under nutrient poor conditions, *C. canescens* has higher photosynthetic rates than *P. arundinacea*, indicating an adaptive advantage. This was confirmed in various studies, which found that conservative species with their longevity, low absorption rates and low growth rates are generally found in nutrient poor habitats, where their low metabolism and high duration are evolutionary favored (Reich et al., 1998; Colesie et al., 2020; Káplová et al., 2011). If nutrient availability would decrease, *C. canescens*' growth rates would only experience a slight drop (Chapin, 1980; Aerts & Chapin, 2000; Kaštovská et al., 2015).

Concerning *P. arundinacea*, high productivity in nutrient-rich environments comes at the cost of longevity, a trait beneficial for survival in low-nutrient conditions due to the tradeoff between productivity and nutrient retention (Aerts & Van der Peijl, 1993; Aerts, 1999)

When comparing P_{max} and regressions (Figure 6) for all species, the following holds true: There is a shift in grouping where plants of the same species had similar photosynthetic behavior in May, while in June plants of the same fertilization treatment performed similarly. These grouping patterns stayed consistent until July. At the beginning of the growing season, species effects were stronger indicators of photosynthetic performance than the nutrient availability, possibly due to the fact that the plants were not yet fully adjusted to the experimental environment. Nutrient uptake in grasses is low in early growth stages (Bruulsema et al., 2016; Hart et al., 1989). As the growing season proceeded, the nutrient environment became the driving factor for productivity, possibly due to higher absorption rates with plant maturity. Even though the absolute Chl and EPS contents varied, these general patterns were consistent when computed on an area vs. DW basis. With this in mind, hypothesis 3 can be partially confirmed. The hypothesis presumes that the species is the main factor determining photosynthetic efficiency. This was just the case in May, but not as the growing season progressed.

Hypotheses 3 and 4 predicted that photosynthetic rates are closely correlated with Chl contents. Overall the regression slopes showed extraordinarily low R squared values, with few exceptions, which is counter to the results of other studies. Buttery and Buzzell (1977), Wang et al. (2020) and Nagaraj et al. (2002) are just a few of many sources which found a close correlation between photosynthesis and Chl. This implies that in our experiment there might have been another factor limiting photosynthesis, even though Chl levels were adequate (Blackman, 1905). Water levels, CO_2 availability, but most importantly irradiation could be influences (Šesták, 1966). Higher correlations between photosynthesis and Chl were obtained when sufficiently high irradiation levels to saturate all tissue layers were applied. However, under too high irradiation, photoinhibition could occur, which is essentially a retardation of photosynthetic reaction centers (Rezai et al., 2018). Another factor which could lead to more consistent correlations was when photosynthesis was related solely to Chl a, instead of Chl a+b content (Šesták, 1966). Additionally, it would be useful to increase the sample size, in order to achieve lower variances and more reliable statistical test results. This especially applies to May and July, where the sample sizes were not sufficient to conduct statistical tests.

When investigating Chl levels, there is a choice between expressing this quantity per unit leaf area or DW. At first, a normalization based on leaf area might be intuitive, as it provides the surface for light interception. However, in this thesis the results were more consistent when expressed on a DW basis. The artificially high Chl levels for *C. canescens* in May are due to the remarkably low leaf areas, which might have been the consequence of a measuring error when handling the scanning software. Alternatively, the SLA could be used as a basis for expressing Chl contents. This would combine area and DW into one quantity.

Lastly, to fully answer hypothesis 1, all experimental results need to be considered together: fertilization seemed to rather affect physiological, rather than morphological parameters. Stem count and height were solely dependent on innate plant type. Although photosynthesis was heavily affected by the species at the start of the growing season, fertilization came to be a more pronounced influence later. This is consistent with the biomass data, which was thoroughly positively affected by fertilization. This suggests that fertilization effects are time dependent, which is common especially in ecosystems with long lived vegetation or rhizomatous plants, where future growth depends on the storage of resources within belowground structures (Güsewell et al., 2002). Regarding the effects of fertilization on chlorophyll, there was no consistent correlation.

Although we predict that the observed results hold true for a time period extending one summer, a long term study might be needed to confirm the consistency of these patterns. Additionally an increase in plant samples would make the results more reliable. In this thesis there were not enough samples in July, so no data analysis could be conducted for this month, thereby greatly reducing the reliability of the results. It would also contribute to the bigger picture if biomass data was available over the whole growing season, not just once at the end.

The obtained results offer valuable insights for managing wetlands invaded by *P. arundinacea* and in invasion prevention, thereby conserving biodiversity. To achieve that, it is advised to keep soil nutrient contents low, creating a non-ideal environment for opportunists like *P. arundinacea* (Wetzel & Van der Valk, 1998). This could be accomplished by applying sawdust, which causes microbial immobilization of N, making it unavailable for plant uptake (Iannone & Galatowitsch, 2008). Zhang et al. (2020) reported on the advantages of introducing parasitic plant species, which reduce fast growing species by using up a part of

the available resources, and therefore preventing the exclusion of slow species (Zhang et al., 2020). Additionally it could be helpful to monitor the surrounding fluxes of nutrients, e.g. when the wetland is in the proximity of fields which are subjected to fertilization treatments (Wetzel & Van der Valk, 1998). There is no universal method for each wetland, and often, these methods have effects on native populations as well, which need to be considered beforehand. Independent of the method(s) chosen, continuous monitoring of the affected wetland is vital (Iannone & Galatowitsch, 2008).

6 Conclusion

The following key insights were gained from our experiment:

1. The effects of fertilization on both species were mixed. It did not notably affect stem production and height. These parameters were mainly determined by species specific growth differences. For the photosynthesis, the species effect was predominant in May. However, fertilization became the main driving factor as the growing season progressed. This is also reflected in the biomass at the end of the season. Therefore hypothesis 1 can be partially agreed with.
2. Considering the biomass in July confirms the assumption that fertilization favors *P. arundinacea* and will increase its growth to a greater extent than that of *C. canescens.*
3. *P. arundinacea* had higher photosynthesis levels in May, agreeing with hypothesis 3. By June however, this "species effect" was diminished. Even though *P. arundinacea* was thoroughly the most productive regarding P_{max} when fertilized, the unfertilized treatment was the least productive. Additionally, *P. arundinacea* Chl was not higher than *C. canescens*'. This is not in agreement with hypothesis 3.
4. Hypothesis 4 can be neglected, as the photosynthesis did not seem to correlate with Chl contents.

One final remark when comparing the growth characteristics of two species is that it is crucial to consider the two species different growth styles and environmental niches. Drawing conclusions based solely on factors like biomass oversimplifies the picture. For example, *P. arundinacea* high productivity might lead one to believe it is the favored species. However in oligotrophic environments, this is not advantageous in the long term, and therefore does not reflect the species survival. Similarly *C. canescens* is not low in productivity because it is in poor health. It is simply employing what it is evolutionary equipped to, to thrive optimally within its specific niche. In this case, low productivity is tied to high survival. Considering the species specific niche is critical for result interpretation.

In the future, it would be helpful to additionally examine different levels of submersion. This could give conclusions about how eutrophication and water levels might influence each other. Another way to continue this study would be to conduct it in the field. This could determine if the results obtained from the mesocosm are consistent. It would take into account various biotic factors, such as competition between these species.

7 Literature

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8 Appendix

Appendix 1: Light compensation point [LCP; µmol m^{-2} s^{-1}] throughout the growing season (May - July) for the four plant- and treatment groups. Values are depicted in means ± SD.

	May	June	July
Carex Unfert	46.70 ± 14.40	13.70 ± 6.27	9.63 ± 5.11
Carex Fert	38.58 ± 24.68	13.35 ± 2.92	15.68 ± 6.82
Phal Unfert	18.54 ± 0.66	14.62 ±3.54	10.17 ± 2.78
Phal Fert	20.06 ± 8.95	12.57 ±1.94	9.10 ± 0.55

Appendix 2: Dark respiration [Rd; µmol m^{-2} s^{-1}] throughout the growing season (May - July) for the four plant- and treatment groups. Values are depicted in means ± SD.

	May	June	July
Carex Unfert	1.51 ± 0.55	0.64 ± 0.30	0.43 ± 0.23
Carex Fert	0.96 ± 0.47	0.75 ± 0.10	0.62 ± 0.08
Phal Unfert	1.28 ± 0.47	0.75 ± 0.23	0.46 ± 0.25
Phal Fert	1.14 ± 0.28	0.68 ± 0.22	0.47 ± 0.20

Appendix 3: The quantum yield [QY; m^{-2} s^{-1}] throughout the growing season (May - July) for the four plant- and treatment groups. Values are depicted in means ± SD.

	May	June	July
Carex Unfert	0.04 ± 0.03	0.05 ± 0.01	0.05 ± 0.01
Carex Fert	0.04 ± 0.03	0.06 ± 0.01	0.04 ± 0.01
Phal Unfert	0.07 ± 0.03	0.06 ± 0.01	0.04 ± 0.01
Phal Fert	0.07 ± 0.03	0.05 ± 0.02	0.05 ± 0.03

Appendix 4: Result of the cluster analysis conducted for all individual plant regressions in May on an area basis [µg cm^{-2}]. The values 0 and 1 represent cluster 0 and 1.

	Cluster
Carex Unfert	1
Carex Unfert	0
Carex Unfert	0
Carex Fert	1
Carex Fert	0
Carex Fert	1
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Fert	0
Phal Fert	0
Phal Fert	0

Appendix 5: Result of the cluster analysis conducted for all individual plant regressions in June on an area basis [µg cm^{-2}]. The values 0 and 1 represent cluster 0 and 1.

	Cluster
Carex Unfert	0
Carex Unfert	0
Carex Unfert	1
Carex Unfert	1
Carex Unfert	0
Carex Unfert	1
Carex Unfert	0
Carex Unfert	1
Carex Unfert	1
Carex Fert	0
Carex Fert	1
Carex Fert	1
Carex Fert	1
Carex Fert	1
Carex Fert	1
Carex Fert	1
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1

Appendix 6: Result of the cluster analysis conducted for all individual plant regressions in May on a DW basis [µg mg^{-1} DW]. The values 0 and 1 represent cluster 0 and 1.

	Cluster
Carex Unfert	0
Carex Unfert	0
Carex Unfert	0
Carex Fert	0
Carex Fert	0
Carex Fert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	1
Phal Fert	0
Phal Fert	0
Phal Fert	1

Appendix 7: Result of the cluster analysis conducted for all individual plant regressions in June on a DW basis [µg mg^{-1} DW]. The values 0 and 1 represent cluster 0 and 1.

	Cluster
Carex Unfert	0
Carex Unfert	0
Carex Unfert	0
Carex Unfert	1
Carex Unfert	0
Carex Unfert	0
Carex Unfert	0
Carex Unfert	0
Carex Unfert	1
Carex Fert	0
Carex Fert	1
Carex Fert	1
Carex Fert	1
Carex Fert	1
Carex Fert	1
Carex Fert	1
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	0
Phal Unfert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1
Phal Fert	1
Phal Fert	0
Phal Fert	1
Phal Fert	1
Phal Fert	1