universität wien

MASTERARBEIT | MASTER'S THESIS

Titel | Title

Metabolite screening of two halophytic plant species, Lepidium cartilagineum and Lotus tenuis, growing in the National Park Neusiedler See-Seewinkel

verfasst von | submitted by

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angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2024

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 833
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Ecology and Ecosystems
Betreut von | Supervisor:	Univ.-Prof. Dipl.-Biol. Dr. Stefanie Wienkoop Privatdoz.

Acknowledgements

My supervisor, Prof. Dr. Stefanie Wienkoop, thank you for allowing me to work on this project and being part of such an exceptional group. Your continuous support and readiness to assist with any questions have been invaluable. I appreciate your patience and making the entire master's project, from sampling to writing, a thoroughly enjoyable experience.

I want to extend my sincere thanks to Dania Randi, for insightful scientific guidance and unwavering support throughout the research. Her direction was crucial in shaping my work, and I truly valued the enjoyable and often fun moments we shared in the lab and during sampling. These memories will be cherished for years to come.

My gratitude also goes to the entire PMInt group for their support and willingness to assist with any questions, which were important for the success of this project. Thank you, Julian Preiner, for adjusting the protocol and always being ready to help. A special thanks to Carlos Pérez Rízquez for his kindness, willingness to share his knowledge, and patient assistance in the lab.

I also want to thank our lab managers, Clara Priemer and Lena Fragner, for their unwavering support and readiness to assist with any laboratory questions that arose.

Additionally, I am grateful to our gardeners, Andreas Schröf and Thomas Joch, for their diligent care of our plants and their consistent friendliness and assistance.

I want to thank my family and friends for their unwavering support and for bringing joy even during the toughest times. A special thanks to Gabriel for being my emotional anchor, providing valuable advice on academic and personal matters, and his assistance with R and mathematical challenges. Your support has made a significant impact throughout this journey.

Abstract

The Neusiedler See–Seewinkel region in Austria is a semiarid area with a Pannonian climate, characterized by hot, dry summers and cold winters. The remnants of the Pannonian Sea have resulted in the development of salt-bearing horizons, resulting to highly saline-alkaline soils Therefore, only species with specialized adaptations can thrive in these challenging conditions. In our study, we analyzed various metabolites—including chlorophyll a, chlorophyll b, carotenoids, total phenols, total flavonoids, soluble sugars, free amino acids, proline, and total proteins—to identify the metabolic and protein-level adaptations that enable these plants to overcome this saline and dry environment. We studied two halophytes, *Lepidium cartilagineum* (Brassicaceae) and *Lotus tenuis* (Fabaceae)—across two seasons (April and September) and sites at Seewinkel: Alte Mühle and Seevorgelände, additionally was *L. tenuis* also sampled at Darscholacke, and Sechsmahdlacke. As a proxy to environmental conditions over two seasons and across sites, this study also provide insight of soil properties, concretely soil water content (SWC), pH ($CaCl_2$), ionic composition (Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, CO_3^{2-}, SO_4^{2-}), and conductivity, present in the soil surrounding the root system (rhizosphere) of studied plants during the sampling time.

We found seasonal and spatial variability in conductivity and ion content; however, the ionic ratio was similar at all sites and for both species. All soils showed to be dominated by sodium and, on the anion side, by carbonate/sulfate/chloride. Both plants experienced different levels of ions and salinity, where soil from *L. cartilagineum* root zone revealed higher salinity and ionic content (Na^+, Cl^-, CO_3^{2-}, SO_4^{2}), whereas *L. tenuis* showed to prefer lower salinity content. *L. cartilagineum* was shown to perform the best in the most saline soils by elevated photosynthetic pigments and proteins. *L. cartilagineum* also accumulated high levels of amino acids, especially proline, even in non-saline environments (control), indicating an inherent osmotic regulation mechanism. Both species showed lower pigment and protein levels in April than in September, likely due to less effective salt tolerance plants must overcome in the early life cycle. For *L. tenuis*, this may also result from less effective symbiosis early in the season. This was particularly evident in plants from Darscholacke, which performed poorly in April but improved by September, aligning more closely with nitrogen-fixing controls. By September, plants showed increased levels of photosynthetic pigments, proteins and reduced variability in sugars and amino acids, indicating a symbiotic adjustment that enhances resilience to salinity later in the season. This is highlighting a potential adaptive mechanism for *L. tenuis* in saline environments. Control plants had higher chlorophyll and carotenoid content, suggesting that wild plants (from Neusiedler See-Seewinkel) reduce pigments under salinity stress, due to their succulence and large vacuoles. However, increase photosynthetic pigments in non-saline environments to improve photosynthesis. Both plants showed elevated content of soluble sugars, phenols, and flavonoids compared to the controls, suggesting that their osmotic and antioxidative adjustment resulted in a salt-tolerance mechanism.

Table of Contents

1 Introduction

In recent years, rising temperatures and increased land use, hence intensified irrigation practices, have subjected plants to higher drought and salinity stress, causing a decrease in crop yield (Adams et al., 1998; Corwin, 2021). Drought and salinity are among the most significant environmental stresses. Rising temperatures and lower precipitation rates are causing higher evaporation, resulting in drought; in an arid environment, this leads to the upward movement of groundwater through capillary action, bringing accumulated ions to the surface (Albert et al., 2020). As of 2022, the United Nations projects that the global population will reach 9.7 billion by 2050 and 10.4 billion by 2100 (United Nations, 2022), creating an urgent need to increase food production. The worldwide increase in soil salinity is attributed not only to primary salinity—caused by natural processes such as rock weathering, ion accumulation, and groundwater movement due to drought—but also to secondary salinity, which results from land degradation caused by poor irrigation practices with salt-rich water and inappropriate fertilizer use. These factors significantly contribute to the reduction of crop productivity (Corwin, 2021; Hailu & Mehari, 2021). Plants can be categorized as either glycophytes or halophytes based on their ability to live and reproduce in saline environments. Halophytes have evolved various biochemical, anatomical, and physiological adaptations to survive in saline conditions, while glycophytes, which include most crop plants, are unable to tolerate high salinity (Grigore et al., 2014a). By studying the adaptations of halophytes, we can gain valuable insights into the traits that enable them to thrive in saline environments. These traits could potentially be introduced into crop plants, allowing them to survive in saline conditions (Flowers et al., 1986; Rahman et al., 2021).

1.1 Study area – Neusiedler See – Seewinkel

Neusiedl Lake (Neusiedler See) is the largest steppe lake in Europe and, with an area of around 315 km^2, is also the overall largest lake in Austria. The lake is located on Austrian-Hungarian borders, situated in an endorheic Pannonian basin, and belongs to Nationalpark Neusiedler See – Seewinkel (Soja et al., 2013). The formation of the lake is dated 13 000 years before the present, by tectonic delve; because of its high wind exposure and shallowness, the lake experienced many dry outs during its existence (Herzig, 2014).

The area surrounding the lake belongs to the warmest, sunniest, and dryest regions in Austria. The Neusiedler See–Seewinkel region is the only semiarid region in Austria. In arid and semi-arid regions, the predominant cations are sodium (Na^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}), while the main anions are bicarbonates and carbonates, contributing to a high soil pH (>8). Magnesium and calcium bicarbonates remain in the soil solution, but as the soil dries and precipitation processes occur, their concentrations decrease, making sodium the most abundant cation (Warrence et al., 2002). The climate

is Pannonian and characterized by dry and hot summers and cold winters. Thus, temperatures fluctuate between 40 °C in the summer and down to -20 °C in the winter. Seewinkel is the area east of the lake, and its landscape is characterized by the occurrence of many typical lakes - salt ponds (Ger. "Salzlacken"), found only here in Europe. These lakes are approximately 70 cm deep, and their number is decreasing every year. Due to their shallowness, they depend on precipitation and are susceptible to desiccation. In the 19th century, there were estimated to be 140 of them, and in 2021, only 59 were reported (Zimmermann-Timm & Teubner, 2021). In a report from 2009 (Eitzinger et al., 2009), measured a yearly average temperature of 10.1°C and precipitation of 574mm at Neusiedler am See station. A recent study from 2023 (Hackl & Ledolter) revealed an average yearly temperature of approximately 13°C in 2020 and an average yearly increase of 0.047 °C (1976-2020). Higher temperatures contribute to the desiccation of salt lakes and higher accumulation of salt in the Seewinkel soil.

The soil at Seewinkel is highly sodic-saline and consists of two types, Solonchak and Solonetz. Solonchak is a white alkali soil where the concentrations of ions decrease with depth. This soil is rich in carbonate, hydrogen carbonate, sulfate, and chloride anions and, on the cation side, predominantly in sodium. Solonchak is distributed throughout the whole area of Seewinkel, predominantly at Seevorgelände, and a typical plant that appears in this soil is *Lepidium cartilagineum*. Solonetz is a second type, a black alkali soil, where the salt-bearing horizon got overlayed by sediment, allowing for continuous vegetation and forming a hummus-rich A-horizon. The soil is also rich in sodium (70%), causing visible polyhedral cracks on the soil surface due to sodium's strong swelling effect (Albert et al., 2020). The presence of both types of soils is due to the salt-bearing horizon in this area, which was formed from the remains of the Pannonian Sea covering this area and leaving behind extensive layers of clays, sand, and gravel. The salt accumulation was due to following environmental conditions that led to the accumulation of sodium salts in a specific clay-sandy channel within the basin. Consequently, water evaporation through the channels led to the concentration of sodium salts and the formation of the initial salt-bearing horizon. With time, windblown loess, rich in calcium carbonate, was deposited on the horizon, and the interaction contributed to the characteristics of the Solonchaks and Solonetz soil types found in the Seewinkel (Häusler, 2020).

1.2 Halophytes, *Lotus tenuis* and *Lepidium cartilagineum*

Salinity and drought are closely linked not only in soil but also as an abiotic stress factor to plants, as high soil salinity limits water uptake by roots, thereby stunting plant growth. Consequently, plants exhibit similar responses to both stresses (Zhu, 2002). High Na^+ concentration in the soil leads to its uptake by roots and accumulation, causing ionic imbalance and disruption of normal plant functions (Kumari et al., 2015). To endure saline conditions, halophytes have developed various mechanisms to

adapt their physiological, morphological, molecular, and biochemical states, allowing them to endure the negative effects of salinity and complete their life cycles (Flowers et al., 1986).

Our study focuses on two halophytes, *Lepidium cartilagineum* and *Lotus tenuis*. We examined the fluctuations in the metabolic concentration of selected metabolites and overall protein content in response to environmental changes in the Seewinkel region, which were studied on the soil parameters level. *Lotus tenuis* is considered a facultative halophyte, as it can be found in non-saline soils, while *Lepidium cartilagineum* is an obligatory halophyte, typically found in highly saline environments (Albert et al., 2020).

Lotus tenuis (*Fabaceae*) is a forage legume inland; it occurs in rough grassland, gravel-, sand-, chalk- and clay pits (Stroh et al., 2023). *L. tenuis* salt tolerance was shown to be due to the ability to exclude Na^+ and Cl^- ions from the shoot when exposed to combined salinity and waterlogging stress(Teakle et al., 2006). Besides, legumes are known for establishing mutualistic symbiosis with soil-borne endophytic rhizobacteria. The symbiosis is initiated by plants when exposed to low nitrogen content in soil by releasing special flavonoids to attract the bacteria. The endosymbiosis forms infection thread and special compartments called nodules. The symbiosis is based on the exchange of nitrogen, provided by bacteria, usually in the form of ammonia, in exchange for energy, in the form of carbohydrates (Mylona et al., 1995). The symbiotic establishment was also proved to be beneficial to plants when exposed to abiotic stresses (Rodriguez et al., 2008). *Lotus tenuis* is, therefore, an important species for studying the salt-tolerance mechanism and its improvement through rhizobacterial symbiosis. *Medicago sativa* (alfalfa) was shown to endure salinity stress when inoculated compared to non-inoculated with rhizobacteria. Inoculated plants overall performed better under salinity conditions; they increased fresh shoot weight, showed lower levels of reactive oxygen species (ROS), and maintained osmotically active metabolites (Y. Wang et al., 2016). Bacterial adaptations to the saline environment are crucial for symbiosis establishment and were shown to improve *Lotus tenuis* fitness when compared to plants inoculated with bacteria from not salt-impacted soils (Sannazzaro et al., 2011).

Lepidium cartilagineum (Brassicaceae) prefers humic clay soils, dry in surface during summer, and soils with high sodium content and high water pH (9-10). *L. cartilagineum* adjusted to the higher salinity by thickening its leaves, increasing water storage tissue, and accumulating salts in large vacuoles, resulting in succulence (Grigore et al., 2014a). Besides, its morphological adaptation and osmotic changes in high concentrations of proline have been reported (Albert et al., 2020). A study on seasonal changes in the content of osmoprotectant and ROS scavengers revealed that *L. cartilagineum* exhibited the highest proline content in comparison to two other Lepidium species and maintained the highest proline content through the whole study period (March-August) (Murakeözy et al., 2003). Hence,

studying a *Lepidium cartilagineum* species can give us an important insight into metabolic adaptations through the different periods, which help the plant reduce the impact of salt stress.

1.3 Study aim

This thesis investigates the impact of seasonal and spatial changes of environmental conditions studied on soil parameters on the concentrations of eight key metabolites—chlorophyll a, chlorophyll b, carotenoids, phenols, flavonoids, soluble sugars, free amino acids, and proline—as well as total protein content in two halophytes, *Lepidium cartilagineum,* and *Lotus tenuis*. We measured these metabolites using spectrophotometry to assess the plants' physiological responses. The environmental conditions were characterized at the soil level, focusing on soil water content, conductivity, pH ($CaCl_2$), and sodium, magnesium, calcium, potassium, chloride, carbonate, and sulfate concentrations across different sampling sites. The sampling sites were divided into salt patches, characterized by visibly higher salt content and drier soil surface, dominated mostly by *Lepidium cartilagineum* (Alte Mühle and Seevorgelände), and lake-side locations near salt ponds (Darscholacke and Sechsmahdlacke).

The study aims to address the following questions:

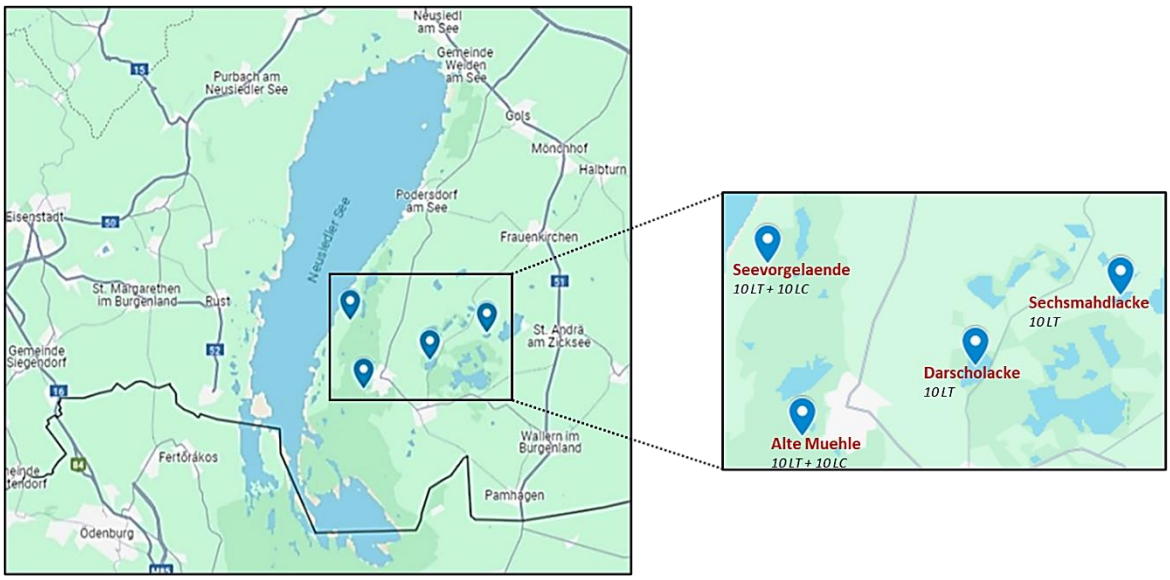
1. What are the seasonal and spatial differences in soil properties across various sampling sites? Do they differ across species?
2. How do plant metabolites and total protein content respond to seasonal and spatial environmental changes?
3. What adaptations have halophytes developed compared to non-halophytic controls, specifically regarding metabolites and total protein content?
4. What are the inherent mechanisms studied halophytes evolved leading to salt-tolerance?

2 Materials and Methods

Plant Material:

Leaves of *Lepidium cartilagineum* and *Lotus tenuis* were collected in Neusiedler See - Seewinkel. Control plants from the same species were grown in a growth chamber condition. Control model species, *Lotus japonicus* and *Lepidium sativum* were also subjected to the same regiment and conditions for comparison.

2.1 Sampling strategy from Neusiedler See – Seewinkel



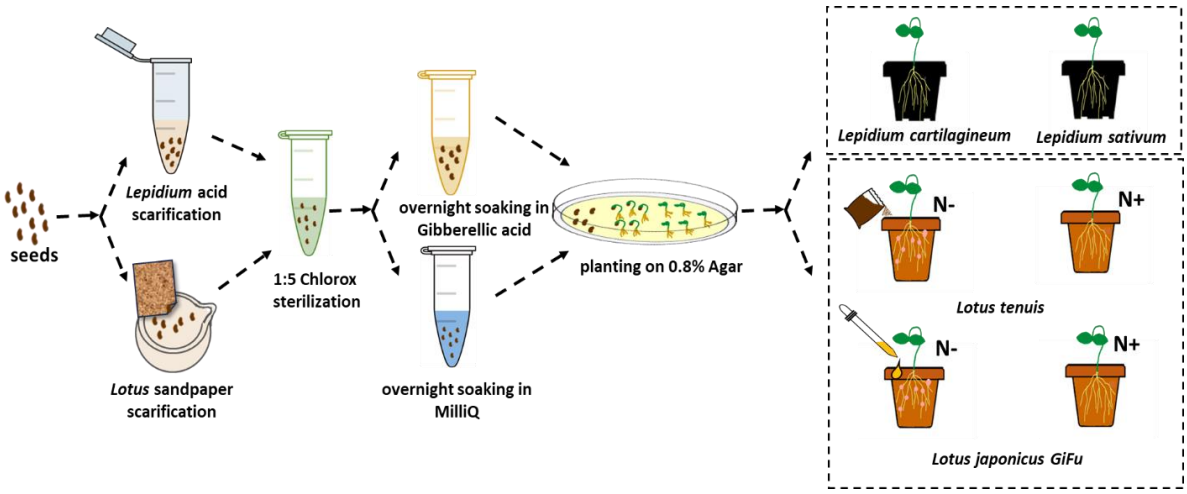
Picture 1: Study area around Neusiedler See - Seewinkel. *Lepidium cartilagineum* (LC), *Lotus tenuis* (LT); 10 representing the number of replicates sampled per each species. Sampling was done during April and September 2022, with random replicates for each species. *L. cartilagineum* was sampled only at Seevorgelände and Alte Mühle, whereas *L. tenuis* was also sampled at Sechsmahdlacke and Darscholacke. All samples were immediately frozen in liquid nitrogen to preserve metabolites and proteins from degrading. We also sampled soil surrounding the root system (rhizosphere) of the first three replicates at each site in both seasons to perform a soil analysis.

In September, a shortage of *Lotus tenuis* individuals led to fewer replicates (6-8). Additionally, an issue with safe-lock Eppendorf tubes was encountered in April. When placed in liquid nitrogen, these tubes exploded due to increased pressure, losing some plant material. This challenge was resolved by using screw-cap tubes in September. Tubes containing the plant material were stored in a -80°C freezer until analysis.

2.2 Measurements of soil parameters

I was provided data on the sampling sites' relevant geochemical soil properties (cation and anion concentrations, pH, electrical conductivity, and soil water content). Soil samples for analysis were collected during the same sampling excursions as plant leaf samples in April and September of 2022. The salinity of the soil pore water, obtained from the saturated soil paste (method described in Black et al., 1983), was characterized through conductivity (ECe) and the concentrations of the main salinity-contributing ions. The concentrations of the mentioned cations and anions were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) and ion chromatography (IC), respectively. Soil pH was measured in 10mM CaCl2 extracts with a soil-to-extractant mass: volume ratio of 1 to 2.5. Soil water content was calculated as the difference between fresh and dry soil weight; soil was dried at 105°C for 24h.

2.3 Control plant material preparation



Picture 2: Experimental design of control plant preparation**.** Seeds of all four species, *L. japonicus*, *L. tenuis*, *L. cartilagineum*, and *L. sativum*, underwent scarification to help them sprout faster. Lepidium plants were scarified using concentrated sulphuric acid, and Lotus underwent manual scarification using sandpaper. All seeds were then treated with Chlorox solution for sterilization. Lepidium species were overnight soaked in Gibberellic acid, and Lotuses were soaked in water. On the next day, all seeds (already open) were planted on Petri plates containing 0.8% agar and left for 3 – 5 days to sprout in dark, warm conditions; after developing the first roots, plants were placed in light conditions until developing the first leaves. Lepidium species were then put into pots containing garden soil. Lotuses were placed into pots containing seramis soaked in B&D solution with low nitrogen concentrations and watered with B&D every second day; half of the plants of *L. japonicus* were further treated with B&D solution containing low nitrogen (0.5mM) concentration and were inoculated with *Mesorhizobium loti* to develop nodules (nitrogen fixing). The second half was treated with a B&D solution containing a

high N+ (5mM) concentration to suppress nodulation (nitrogen-fed). *L. tenuis* was treated in such a way, but instead of inoculating them with bacteria, we sprinkled some of their original soil from Darscholacke (April) into their pots.

2.3.1 Seed scarification, sterilization of control plants

Seeds of *Lepidium cartilagineum* and *Lepidium sativum* initially underwent scarification using concentrated sulfuric acid (H_2SO_4) for 1 minute. Subsequently, the acid was decanted, and the seeds were transferred into clean 2 mL tubes. The seeds were washed six times with PBS solution to remove residual acid while kept on ice throughout the scarification process. For sterilization, a bleach treatment was performed, in a 1:5 solution of 2.8% Na-hypochlorite solution (Chlorox) to water. After a brief vortex, the seeds were incubated for 5 minutes. The seeds were then washed six times with MilliQ water. The prepared seeds were subsequently soaked in 0.1mM Gibberellic acid overnight. Seeds of *Lotus tenuis* and *Lotus japonicus* (Gifu) underwent a scarification process using sandpaper. Subsequently, they were sterilized in a 1:5 solution of 2.8% Na-hypochlorite solution (Chlorox) to water for 15 minutes. They were then washed with MilliQ water six times and soaked in MilliQ water overnight.

2.3.2 Control plants growing conditions

Seeds were placed on petri plates with agar and incubated in dark conditions until sprouting. Afterward, they were placed in light conditions until the first leaves and shoots were developed (3-5 days). Lepidium plants were planted into garden soil - mixed with quartz sand and perlite with a ratio of 7:1:1, with pH 5-6.5; salt content (KCl) 3 g/L, Nitrogen (N) content 200-500 mg/L; Phosphate (P_2O_5) 200-1000 mg/L and Potassium (K_2O) 400-1400 mg/L and growing for ~60 days. Lotus plants were growing in seramis. Seramis soaked in a B&D solution with 0.5 mM KNO3 concentration for both species. After a week, all plants were treated with a B&D solution containing a higher concentration of KNO_3 (5 mM) to avoid nodulation. After a week, *Lotus japonicus* were inoculated with ca. 10 mL of *Mesorhizobium loti* R7A strain. *L. tenuis* was inoculated by spreading their original soil from Darscholacke on pots and watered with distilled water for better penetration. When plants were ready for sampling, leaves were cut and placed into screw cup tubes and immediately put into liquid nitrogen and stored until the analysis. Conditions in the growth chamber were similar to those in April. Daily temperature 22°C and humidity 50% and night temperature 17°C with humidity 65%, with photoperiod 14 hours with photosynthetically active radiation set to 400 µmol/m²/s during the day.

2.3.3 Bacterial growth of *Mesorhizobium loti*, R7A strain

On the first day, 150 µL of the bacterial culture *M. loti* R7A was spread on Yeast Extract Mannitol (YEM) agar plates and incubated over the weekend. Following this, the growing culture was resuspended in 45 mL of Tryptone-Yeast Extract medium (TY) and allowed to grow at 28°C for two days. Subsequently, 20 mL of the bacterial solution was divided into 185 mL of fresh YEM media and another 20 mL into 185 mL of fresh TY media, maintained at 28°C and 100 rpm for two days. The media from both cultures were pooled, divided into 50 mL Falcon tubes, and centrifuged at 4000 rpm for 10 minutes. The remaining supernatant was discarded, and the pellet was resuspended in deionized water. This prepared suspension was used for the inoculation of *L. japonicus* plants.

2.4 Sequential extraction and estimation of metabolites and total protein content

For further metabolite analysis, plants were freeze-dried. Freeze drying was done in a minimum of three days. The dried leaves were subsequently manually ground to powder in a ceramic mortar. A dried weight corresponding to 60 mg of fresh weight was taken for further analysis. The extraction of photosynthetic pigments and preparation of the metabolites pool were executed according to Lichtenthaler & Wellburn (1983). For further metabolite extraction, a Sequential extraction protocol was adjusted and used (Laware, 2015).

Chemicals: all metabolites were extracted using chemicals from Sigma Aldrich Handels GmbH.

Extraction of photosynthetic pigments

Photosynthetic pigments assay was executed according to Lichtenthaler & Wellburn (1983) using the acetone method. To the tubes containing the corresponding weight of powdered samples, 1 mL of prechilled 80% acetone was added, thoroughly vortexed, and centrifuged at 10,000 x g for 10 minutes. The supernatant was collected into reagent tubes. The pellet was reextracted with 0.5 mL of 80% acetone and centrifuged again, repeating this step twice. All supernatants were collected into the same tube, resulting in approximately 2 mL of acetone extract. Both pellets and supernatants were kept on ice throughout the entire extraction process. Photosynthetic pigments were measured in triplicates from the acetone extract. The extract was five times diluted; 50 µL of the extract was applied directly to a 96-well plate containing 200 µL of 80% acetone. As a blank, only 80% acetone was used. The samples were measured at 663, 646, and 470 nm wavelengths.

Extraction of soluble carbohydrates, total phenol content, flavonoids, free amino acids, and proline using methanol & and ethanol extraction

After acetone extraction, the residue was used to extract all remaining metabolites for further analysis. In this step, the pellet was first re-extracted with 1 mL of pre-chilled 80% methanol, vortexed, incubated at 95°C for 30 minutes, afterward, cooled on ice, vortexed again, and centrifuged at 20,000 g for 10 minutes. The supernatant was collected into a 2 mL tube and repeated using pre-chilled 80% ethanol. Following methanol and ethanol extraction, the pellet was dried under a fume hood and stored at -80°C until used for protein analysis. All three phases were pooled together for further metabolite extraction in a 1:0.5:0.5 ratio of acetone, methanol, and ethanol phases, resulting in a 2 mL mixture. This mixture was then employed to extract soluble sugars, phenols, flavonoids, free amino acids, and proline.

Extraction of soluble carbohydrates

The assay was done according to Hansen & Møller (1975) using the anthrone method. Extraction was performed with 100 µL of the sample pool, standards, and blank mixing with 200 µL of pre-chilled 72% sulfuric acid and vortexed. Subsequently, 400 µL of Anthrone reagent (10g/1L conc. H_2SO_4) was added, vortexed, and the mixture was incubated at 95°C for 15 minutes. Each sample's background was prepared by mixing 100 µL of the samples with 72% sulfuric acid. A 5mM Glucose solution (in 80% ethanol) was diluted in 72% sulfuric acid to concentrations of 50, 250, 500, 1000, 2000, and 3000 nmol/mL to prepare standard curves. As a blank MilliQ, water was used. After incubation, the samples, blanks, and standards were cooled and vortexed, and 250 µL of the samples were loaded onto a 96-well plate in duplicate. Absorbances were measured at 630 nm.

Extraction of phenols

Estimation of total phenol content was conducted following John et al. (2014) using the Folin-Ciocalteu method, 100 µL of the sample pool, standards, and blank were diluted in 700 µL of MilliQ water. Subsequently, the samples were mixed with 80 µL of Folin-Ciocalteu phenol reagent (2 N) and incubated for 5 minutes at room temperature. Then, 800 µL of a 7% Na2CO3 solution was added to the mixture, stirred, and incubated for 90 minutes at room temperature. A standard curve was prepared using diluted Gallic acid in water at 20, 40, 60, 80, and 100 µg/mL concentrations. After incubation, samples, standards, and the blank (MilliQ) were loaded onto a 96-well plate with 250 µL in duplicates, and the absorbance was measured at 550 nm.

Extraction of flavonoids

Total flavonoid content was estimated, and the protocol was adapted following (Chang et al., 2002) and adjusted by (John et al., 2014) using the aluminium chloride ($AlCl_3$) method; 100 µL of pooled samples/standards and blank with 900 µL of MilliQ water. 60 µL of 5% NaNO2 solution was added to the diluted samples, thoroughly mixed, and incubated for 5 minutes. Subsequently, 60 µL of 10% $AlCl_3$ was pipetted into the mixture, shook, and after 5 minutes, the mixture was combined with 400 µL of 1 M NaOH solution, mixed, and topped up with 480 µL of MilliQ water to a final volume of 2 mL. Quercetin stock solution (1g/L in 50% Ethanol) was used as a standard in concentrations of 20, 40, 60, 80, and 100 µg/mL; MilliQ water was used for the blank and standard dilutions. Samples, blanks, and standards were loaded in duplicates at 250 µL each onto a 96-well plate and measured at 510 nm.

Extraction of free amino acids

Extraction of free amino acids was conducted following Moore & Stein (1948) using ninhydrin method. For the extraction, 200 µL of the sample, blank, and standards were combined with 100 µL of 2% Ninhydrin reagent, gently mixed, and heated at 95°C for precisely 10 minutes. Afterward, the mixture was rapidly cooled to room temperature, mixed with 500 µL of 95% ethanol, and vortexed. Measurements were taken using 250 µL in duplicates. Standards were prepared using a 500 µM L-Lysine dissolved in 0.05% Acetic acid, diluted in MilliQ water to 100, 200, 300, 400, and 500 µM, and measured in duplicates at 570 nm.

Extraction of proline

Proline was extracted using the same procedure with some adjustments. The assay was conducted following the method by (Moore & Stein, 1948) and further adjusted according to (Bates et al., 1973) and (Carillo & Gibon, 2011). Standards were prepared using a 5000 µM L-Proline stock, diluted in 0.05 acetic acid, with concentrations ranging from 100 to 5000 µM. Initially, standards were set at 100 to 500 µM concentrations, adjusted to 1000 µM. Later, to align with absorbance values within the range of *Lepidium cartilagineum*, concentrations were adjusted to 5000 µM. The blank consisted of a mixture containing acetone, methanol, and ethanol with the same concentrations and ratio as in the samples, as it produced significantly higher absorbances than MilliQ water. Absorbances for estimating proline content were measured in duplicates at 520 nm.

Extraction of total proteins

Bradford assay was used to estimate the total protein content, according to Bradford (1976) using Bradford method. Pellets from the previous steps were firstly dissolved in 60 µL of solubilization buffer

(0.8 MUrea buffer, 100 mM AmBic), incubated for 30 minutes on ice and for an additional 5 minutes at room temperature, and centrifuged at 20 000 g for 5 minutes. As a blank, Bradford reagent was used as a standard 1 mg/mL. Bovine Serum Albumin was diluted in water and used in dilutions of 1, 2, 3, 4, and 5 µg/mL. Samples were diluted to a ratio of 1:10 in 10 µL of Solubilization buffer and directly loaded on 96-well plates containing 250 µL of Bradford reagent in triplicates. Absorbances were measured at 595 nm.

2.5 Statistical analysis

One-way ANOVA and Tukey HSD to identify seasonal and spatial differences in examined soil properties

A one-way ANOVA and Tukey-HSD post hoc tests were conducted to identify significant differences in soil properties, concentration of metabolites, and total proteins between compared groups across both species. Overall, for soil properties, each site and season 24 replicates were analyzed from *Lotus tenuis* soil and 12 from *Lepidium cartilagineum* soil.

Principal component analysis (PCA), one-way ANOVA with Tukey HSD, to identify effects of seasonal and spatial separation on metabolites and total protein content

We experienced a data loss in April due to the explosion of Eppis in liquid nitrogen. In September, at site Alte Mühle, we lost one replicate; and fewer plant species of *L. tenuis* were present and sampled. Extreme outliers (very high or very low concentration across more than one metabolite) were excluded using the IQR method. For *Lotus tenuis*, in April, we detected two, and in September, four outliers, with final replicates 70 across four sites and two seasons. For *Lepidium cartilagineum*, an extreme outlier was detected in the first three replicates of protein concentration sampled in September at the Seevorgelände site. However, this was not deleted to keep sufficient replicates for soil properties analysis. This outlier was high in protein content (**Table S29**). Overall, 40 replicates were analyzed across two sites and two seasons for natural samples of *Lepidium cartilagineum.* One-way ANOVA with Tukey-HSD post hoc test was conducted to identify significant differences. For principal component analysis, the outliers were deleted, and data were standardized.

Redundancy analysis (RDA), correlation between soil properties and metabolites

An RDA was conducted for soil properties data to detect the correlations between soil properties and chosen metabolites. We determined ten soil properties were soil water content (SWC), calcium chloride soil pH measured in a calcium chloride extract (pH ($CaCl_2$)), soil pore water conductivity (ECe), the concentration of sulfates (SO_4^{2-}) and carbonates (CO_3^{2-}) and concentration of, magnesium (Mg^{2+}),

sodium (Na^+), calcium (Ca^{2+}), chloride (Cl^-) and potassium (K^+) ions. Before RDA, metabolite data from the *Lotus tenuis*, first replicate from the Alte Mühle site collected during September, was imputed due to sample loss. Imputation was performed using a mean metabolite concentration of the remaining replicates (n = 5). Data on soil properties were not imputed, as an adequate number of replicates was collected and analyzed. RDA was first conducted for both species to find soil properties that contributed to variation in analyzed metabolites the most. To see the best explanatory variables (soil properties) for the dependent variables (plant metabolites), a stepwise variable selection algorithm, with afterward a multicollinearity test – variance inflation factor (VIF), with threshold value 20, was conducted. Since the soil properties and metabolite concentrations differed across all sites for both species, a different set of soil properties was identified with the best explanatory variables for each species. It is important to note that soil ion content and conductivity were highly correlated, and only the least correlated variables that contributed to explained variance were chosen. SWC, pH, and conductivity were used for both species with differing ions. The significance of each variable was done using permutational ANOVA (PERMANOVA) with permutation = 1000.

Comparison of controls and natural samples using one-way ANOVA with Dunnett's test and PCA

Compared were all controls to wild species from Seewinkel. Overall, we analyzed eight replicates for control species. IQR method was used to eliminate outliers in nitrogen-fed *Lotus japonicus* samples (n = 6) and *Lepidium sativum* (n = 7). Controls and wild species were analyzed in one dataset. To gain the overall differentiation and relative correlation of metabolites to the species, we first conducted PCA, before which data were scaled and centered. Afterward, natural samples were compared to controls for each metabolite using one-way ANOVA and Dunnett's test.

3 Results

Soil properties were collected from the soil surrounding the root systems of three plant specimens of *Lepidium cartilagineum* and *Lotus tenuis* species at each site: Alte Mühle, Darscholacke, Sechsmahdlacke, Seevorgelände, Lepidium was sampled only at Alte Mühle and Seevorgelände, across seasons April and September. In total, ten soil parameters were analyzed (**Table 1**).

Table 1: Abbreviations, meaning, and metric unit of all analyzed soil parameters.

SOIL PROPERTIES		
Abbreviation	**Meaning**	**Metric Unit**
SWC	Soil water content	[%]
pH $CaCl_2$	pH measured in calcium chloride extract	pH
Conductivity	soil pore water conductivity	Ece [mS/cm]
SO_4^{2-}	Sulfate concentration	[mM/L]
CO_3^{2-}	Carbonate concentration	[mM/L]
Cl^-	Chloride concentation	[mM/L]
Mg^{2+}	Magnesium concentration	[mM/L]
Na^+	Sodium concentration	[mM/L]
Ca^{2+}	Calcium concentration	[mM/L]
K^+	Potassium concentration	[mM/L]

3.1 Soil profile characterization of sampling sites across both seasons

This analysis aimed to characterize soil profiles across sampling sites in April and September. To evaluate seasonal and spatial differences, one-way ANOVA followed by Tukey HSD posthoc tests with a 95% confidence interval were conducted. Data were divided into subsets for targeted comparisons. Seasonal differences were analyzed within each site across both seasons, and spatial differences were assessed across all sites within each specific season. Data are represented in a heatmap summary table (**Table 2,3**).

3.1.1 *Lotus tenuis*: comparative analysis of examined soil parameters

soil water content (SWC), pH ($CaCl_2$)

No significant seasonal changes were observed at the Alte Mühle (AM) site for measured soil properties (**Table 2**). **Soil water content** was relatively steady across seasons and sites, with a minimum of 7 ± 0.5 % SD at Sechsmahdlacke (SML) and a maximum of 13 ± 2.5%.

SD at Darscholacke (DL) in April. In September was, the range little bit higher, with the lowest SWC found in the soil from Darscholacke (11 ± 3.9%), and the highest was measured in the soil from Alte Mühle and Seevorgelände (SV) (17 ± 1.1%) (**Table 2**). The **pH of calcium chloride ($CaCl_2$)** was alkaline across all sites and seasons, ranging from 7.7 ± 0.035 at SML to 8.08 ± 0.15 at DL in April. In September, the highest pH was still detected at DL (8.27 ± 0.154) and significantly higher than at SV, with the lowest pH (7.88 ± 0.13) ($p < 0.05$). Seasonally, the pH changed the most at the Sechsmahdlacke site by increasing by 1.1-fold to September (8.18 ± 0.192) ($p < 0.05$) (**Table 2**).

conductivity, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cl^-, CO_3^{2-}, SO_4^{2-}

The concentrations of ions were found in higher concentrations in April at DL than in September (except Ca^{2+}; $p < 0.05$). Generally, the DL site in April revealed the highest seasonal and spatial differences from all other sites. Moreover, it was significantly higher in conductivity, contents of sulfates, chloride, calcium, and magnesium than all other sites in April ($p < 0.05$) (**Table 2**). Further, all soils were rich in sodium on the cation side and carbonate on the anion side, except DL soil in April, which exhibited higher sulfate content. Content of calcium, magnesium, and potassium were exhibiting the least abundant in soils (**Table 2, Table S12**).

Soil pore water conductivity changed seasonally and spatially more in April; the highest conductivity was measured at the DL site (5688 ± 1904 µS/cm) and lowest at the SV site (883.07 ± 224.25 µS/cm); DL was significantly higher than all other sites in April ($p < 0.05$). It was also 4.56-fold higher than DL in September, while the opposite was observed at SV conductivity increased from April to September by 2-fold ($p < 0.05$) (**Table 2**). In September, the sites had similar conductivity values and showed lower variability. The highest conductivity was observed at the SML site (1991.33 ± 394.22 µS/cm), while the lowest was recorded at AM (862.47 ± 234.97 µS/cm) (**Table 2**).

Sodium (Na^+) levels changed seasonally only for soil from DL; from April to September, they decreased by 5.4-fold. The highest content overall was detected at DL in April, with 36.19 ± 5.79 mM/L. Spatial differences were found to be higher in September, with the highest content found at SML (17.45 ± 3.34 mM/L), significantly higher than AM (6.16 ± 3.9 mM/L) and DL (6.76 ± 4.85 mM/L) in September ($p < 0.05$).

Magnesium (Mg^{2+}) concentration was across all sites, except DL, below 1 mM/L. The most significant seasonal change was at DL, from April (5.29 ± 2.18 mM/L) to September (1.37 ± 0.73 mM/L), decreased by 3.9-fold ($p < 0.05$) (**Table 2**). The magnesium concentration fluctuated less in September and changes were seasonally less visible at AM, SML, and SV (**Table 2**).

The concentration of **potassium (K^+)**, in general, decreased from April to September across all sites, with the highest differences at SML and DL by 2.8 and 2.3-fold ($p < 0.05$). The highest K^+ content was measured at SV in April (0.54 ± 0.18 mM/L); as well as in September (0.52 ± 0.11 mM/L), which was found to be significantly higher than all other sites ($p < 0.05$) (**Table 2**).

Calcium (Ca^{2+}), similarly to magnesium, was across all sites found to be at a concentration lower than 1 mM/L, except DL in April (1.88 ± 1.03 mM/L), which was spatially also higher than all other sites ($p < 0.05$). In September, the content of Ca^{2+} remained the highest at DL, which from April decreased to 0.76 ± 0.31 mM/L; seasonal changes in the content of Ca^{2+} were less pronounced and no significant changes were detected.(**Table 2**).

The concentration of **Chloride (Cl-)** were more spatially pronounced in April and seasonally at AM and DL sites. In April the highest Cl^- content was at Darscholacke (8.71 ± 2.67 mM/L), significantly higher than all other sites, and also 7.6-fold higher than DL in September ($p < 0.05$). In September, the content of Cl^- did not exceed 3 mM/L and was the lowest at AM (0.61 ± 0.25 mM/L), lower than in April by almost fourfold (**Table 2**). Sites SML and SV had relatively similar contents of Cl^- across both seasons.

Carbonate (CO_3^{2-}) was more fluctuating in the soil in September and had similar content in the soil in April, ranging from 5.16 ± 1.1 mM/L (AM) to 6.61 ± 4.65 mM/L (SV). In September, the concentrations ranged from 2.56 ± 0.97 mM/L (DL) to 6.16 ± 0.13 mM/L at SV, significantly higher than AM and DL ($p < 0.05$); followed by SML (5.6 ± 0.54 mM/L) with substantially higher concentration than DL ($p < 0.05$) (**Table 2**). Seasonally, a decrease from April to September was observed at AM, DL, and SV and an increase at SML. Significantly at DL by 2.2-fold ($p < 0.05$) (**Table 2**).

Sulfates (SO_4^{2-}) similar to conductivity, Mg^{2+}, Ca^{2+}, and Cl^-, were found in significantly higher concentration in soil from DL (15.72 ± 3.14 mM/L), compared to all other sites and 6.3-fold higher than in September at DL ($p < 0.05$). The lowest sulfate content was measured at AM (0.21 ± 0.06 mM/L) in September and, in April, at SML (0.87± 0.01 mM/L), which increased to September by 2.6-fold ($p < 0.05$). Lower variation across seasons was observed in September (**Table 2**).

Table 2: Summary of the descriptive statistics of measured soil properties in 2022, during April and September. The soil was sampled from the areas where *L. tenuis* plants grow. The table presents mean values, standard deviations, and a compact letter display depicting significant groups based on the Tukey HSD posthoc test; n = 3.

Site	Alte Mühle		Darscholacke		Sechsmahdlacke		Seevorgelände	
Season	April	September	April	September	April	September	April	September
Soil Properties	Mean and (Standard deviation) TukeyHSD seasonally, spatially							
SWC[1]	0.12 (0.002) a,*a*	0.17 (0.075) a,*a*	0.13 (0.026) a,*a*	0.11 (0.039) a,*a*	0.07 (0.005) a,*a*	0.15 (0.066) a,*a*	0.12 (0.088) a,*a*	0.17 (0.011) a,*a*
pH ($CaCl_2$)	7.92 (0.168) a,*a*	7.94 (0.06) a,*ab*	8.08 (0.15) a,*a*	8.27 (0.154) a,*a*	7.7 (0.035) b,*a*	8.18 (0.192) a,*ab*	7.89 (0.229) a,*a*	7.88 (0.13) a,*b*
conductivity[2]	1692.13 (1371.95) a,*b*	862.47 (234.966) a,*a*	5688 (1904.309) a,*a*	1249.4 (750.676) b,*a*	1483.67 (131.089) a,*b*	1991.33 (394.22) a,*a*	883.07 (224.249) b,*b*	1780.67 (272.148) a,*a*
Sodium[3]	15.07 (10.835) a,*a*	6.16 (3.897) a,*b*	36.19 (5.787) a,*a*	6.76 (4.845) b,*b*	12.56 (1.391) a,*a*	17.45 (3.335) a,*a*	16.93 (16.2) a,*a*	14.83 (2.206) a,*ab*
Magnesium[3]	0.31 (0.041) a,*b*	0.53 (0.471) a,*a*	5.29 (2.173) a,*a*	1.37 (0.734) b,*a*	0.3 (0.039) a,*b*	0.25 (0.111) a,*a*	0.48 (0.275) a,*b*	0.4 (0.082) a,*a*
Potassium[3]	0.29 (0.112) a,*a*	0.19 (0.056) a,*b*	0.5 (0.136) a,*a*	0.22 (0.079) b,*b*	0.22 (0.039) a,*a*	0.08 (0.056) b,*b*	0.54 (0.183) a,*a*	0.52 (0.105) a,*a*
Calcium[3]	0.26 (0.035) a,*b*	0.5 (0.285) a,*a*	1.88 (1.025) a,*a*	0.76 (0.312) a,*a*	0.32 (0.051) a,*b*	0.21 (0.04) a,*a*	0.31 (0.069) a,*b*	0.31 (0.055) a,*a*
Chloride[3]	2.39 (3.266) a,*b*	0.61 (0.246) a,*a*	8.71 (2.661) a,*a*	1.15 (1.034) b,*a*	1.83 (0.413) a,*b*	2.83 (1.17) a,*a*	1.73 (1.598) a,*b*	2.3 (1.058) a,*a*
Carbonate[3]	5.16 (1.091) a,*a*	3.69 (1.069) a,*bc*	5.44 (1.464) a,*a*	2.56 (0.971) b,*c*	5.21 (0.69) a,*a*	5.6 (0.542) a,*ab*	6.61 (4.646) a,*a*	6.16 (0.125) a,*a*
Sulfate[3]	1.9 (2.737) a,*b*	0.21 (0.064) a,*a*	15.72 (3.136) a,*a*	2.48 (2.04) b,*a*	0.87 (0.014) b,*b*	2.22 (0.74) a,*a*	2.05 (3.075) a,*b*	1.07 (0.522) a,*a*

[1]percentage (decimal), [2]Ece [µS/cm], [3][mM/L]

Lowest — Highest

Note: **Table 2** The letters indicate seasonal and spatial differences. The first letter(s) represents seasonal differences, while the second letter(s) indicates spatial differences in italics, with April comparisons bolded. Soil property values are color-coded: darker blue indicates higher values, and white indicates the lowest values, with all sites across both seasons considered.

3.1.2 *Lepidium cartilagineum*: comparative analysis of examined soil properties

When comparing sites based on the presence of specific species, soils from locations where *Lepidium cartilagineum* plants grow (**Table 3**) showed higher levels of ions (Na^+, Cl^-, CO_3^{2-}, SO_4^{2-}) and conductivity compared to locations where Lotus tenuis grow (**Table 2**). The most significant difference was observed in sulfate content, which was about a hundred times higher in September in soils from Alte Mühle where *L. cartilagineum* was present (20.608 ± 6.92 mM/L) compared to the soil at Alte Mühle where *L. tenuis* was growing (0.21 ± 0.06 mM/L).

soil water content (SWC), pH ($CaCl_2$)

Overall, **soil water content (SWC)** was consistent across all sites and seasons, being higher in September and spatially higher at SV compared to AM during both seasons. The highest SWC levels were recorded in September at SV (16.4 ± 7.8%) and the lowest at AM in April (14.2 ± 1.1%) (**Table 3**). The **pH ($CaCl_2$)** levels were generally alkaline, ranging from the weakest at SV in April (8.123 ± 0.08), which was significantly lower than at AM (8.387 ± 0.14) and seasonally lower than pH at SV in September (8.84 ± 0.39) ($p < 0.05$) (**Table 3**).

conductivity, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cl^-, CO_3^{2-}, SO_4^{2-}

Overall, no significant seasonal changes were observed in conductivity and ions content, additionally, sodium and magnesium content also did not changed spatially. Predominant ion ction in all soils was sodium, as anion was predominant carbonate at SV, at AM we found similar contents of carbonate, chloride and sulfate (**Table 3,** ***Table S19***).

Conductivity and **sodium (Na^+)** contents were higher at SV than at AM across both seasons (**Table 3**). The highest conductivity and Na^+ were at SV in September (15510 ± 4022.39 µS/cm and 169.243 ± 49.37 mM/L, respectively) and the lowest at AM in September (10442 ± 2745.15 µS/cm and 101.07 ± 30.45 mM/L, respectively) (**Table 3**). **Mg^{2+}** was overall higher at AM across both seasons, and the highest Mg^{2+} was found to be in September AM (0.101 ± 0.04 mM/L) and the lowest concentration in April SV (0.035 ± 0.009 mM/L) (**Table 3**).

Potassium (K^+) concentrations were the highest at the SV site in April and September (2.088 ± 2.12 mM/L; 2.02 ± 0.4 mM/L) compared to AM (0.945 ± 0.08 mM/L; 0.838 ± 0.18 mM/L, respectively) (**Table 2**) ($p < 0.05$). **Calcium (Ca^{2+})** was higher at AM across both seasons, with the highest content in September (0.108 ± 0.05 mM/L), which was almost fourfold the content measured at SV 0.029 ± 0.008 mM/L). Significantly was the content of calcium higher than the content at SV in April (0.021 ± 0.002 mM/L) ($p < 0.05$). Generally, calcium content increased from April to September in both sites (**Table 3**).

The concentration of **chloride (Cl^-)** was the highest at AM in April (25.58 ± 2.17 mM/L) and lowest at SV in April (14.612 ± 1 mM/L). In general, was the chloride concentration higher in AM soil during both seasons, significantly in April ($p < 0.05$) (**Table 3**).

The concentration of **carbonate (CO_3^{2-})** was significantly higher at SV across both seasons ($p < 0.05$). The bigger differences were detected in September, differing by threefold (52.195 ± 8.81 mM/L at SV and 29.719 ± 6.13 mM/L at AM) (Table 3).

The concentration of **sulfate** (SO42-), similar to Cl-, was significantly higher at AM in April (27.906 ± 3.58 mM/L) and lowest at SV April (13.6 ± 2.76 mM/L) ($p < 0.05$). In September, the sulfate content remained higher at AM (20.608 ± 6.92 mM/L) than at SV (18.112 ± 6.59 mM/L).

Table 3: Summary statistic of measured soil properties from the soil where L. cartilagineum plants grow. The table presents mean values, standard deviations, and a compact letter display depicting significant groups based on the Tukey HSD post-hoc test; n = 3.

Site	Alte Mühle		Seevorgelände	
Season	April	September	April	September
Soil Properties	Mean and (Standard deviation) TukeyHSD seasonally, spatially			
SWC[1]	0.142 (0.011) a,***a***	0.157 (0.027) a,*a*	0.156 (0.037) a,***a***	0.164 (0.078) a,*a*
pH ($CaCl_2$)	8.387 (0.138) a,***a***	8.773 (0.394) a,*a*	8.123 (0.081) b,***b***	8.84 (0.392) a,*a*
conductivity[2]	12903.333 (1466.47) a,***a***	10442 (2745.154) a,*a*	13873.333 (2020.528) a,***a***	15510 (4022.387) a,*a*
Sodium[3]	139.667 (17.842) a,***a***	101.07 (30.452) a,*a*	144 (22.928) a,***a***	169.263 (49.372) a,*a*
Magnesium[3]	0.057 (0.012) a,***a***	0.101 (0.041) a,*a*	0.035 (0.009) a,***a***	0.04 (0.007) a,*a*
Potassium[3]	0.945 (0.077) a,***b***	0.838 (0.184) a,*b*	2.088 (0.212) a,***a***	2.02 (0.4) a,*a*
Calcium[3]	0.052 (0.01) a,***a***	0.108 (0.05) a,*a*	0.021 (0.002) a,***b***	0.029 (0.008) a,*a*
Chloride[3]	25.58 (2.169) a,***a***	21.779 (4.817) a,*a*	14.612 (1.002) a,***b***	16.4 (3.944) a,*a*
Carbonate[3]	29.719 (6.126) a,***b***	19.665 (6.66) a,*b*	52.195 (8.811) a,***a***	59.398 (17.065) a,*a*
Sulfate[3]	27.906 (3.576) a,***a***	20.608 (6.916) a,*a*	13.6 (2.759) a,***b***	18.112 (6.595) a,*a*

[1]percentage (decimal), [2]Ece [µS/cm], [3][mM/L]

Lowest — Highest

Note: **Table 3** presents the means and standard deviations of soil properties from the soil surrounding the *Lepidium cartilagineum* root zone. The letters indicate seasonal and spatial differences. The first letter(s) represent seasonal differences, while the second letter(s) indicate spatial differences in italics, with April comparisons bolded. Soil property values are color-coded: darker blue indicates higher values, and white indicates the lowest values, with all sites across both seasons considered.

3.2 Metabolite profiling of halophytes: *Lotus tenuis* and *Lepidium cartilagineum* from Neusiedler See-Seewinkel

The objective of this analysis was to investigate changes in metabolites and total protein content across various sampling sites over two seasons from *Lotus tenuis* and *Lepidium cartilagineum* leaves extract. Each plant species was examined independently. Overall differences in metabolites and proteins were first assessed using principal component analysis (PCA) to gain an overview of the most substantial separation effect (**Figure 1, 5**). To enable targeted seasonal and spatial comparisons, the data were divided by site and season and analyzed using one-way ANOVA, followed by Tukey HSD post hoc tests with 95% confidence interval and represented in boxplots (**Figure 2-4, 6-8**). Metabolites were collected from 8 to 10 specimens from Seewinkel. Overall, 8 metabolites and total protein content were analyzed from the plants' leaves of *Lotus tenuis* and *Lepidium cartilagineum* (**Table 4**).

Table 4: Abbreviations, meaning and metric units of all analyzed metabolites and total protein content.

METABOLITES		
Abbreviation	**Meaning**	**Metric Unit**
ChlA	Chlorophyll a concentration	[µg/mg DW]
ChlB	Chlorophyll b concentration	[µg/mg DW]
Carot	Carotenoids concentration	[µg/mg DW]
Phe	Phenols concentration	[µg/mg DW]
Flav	Flavonoids concentration	[µg/mg DW]
SS	Soluble Sugars concentration	[nmol/mg DW]
AA	Free Amino Acids concentration	[µmol/mg DW]
Pro	Proline concentration	[µmol/mg DW]
Prot	Proteins concentration	[µg/mg DW]

3.2.1 Overall metabolites profile of *Lotus tenuis*

The principal component analysis (PCA) (**Figure 1**) explained 58.8% of the variance observed across seasons and sites, with PC1 accounting for 36.8% and PC2 for 21.95%. Seasonal differences were more apparent on PC2, while PC1 primarily captured spatial differences. Notably, in April, Alte Mühle and Darscholacke metabolites clustered together, distinctly separate from those at Sechsmahdlacke and Seevorgelände along PC1 (**Figure 1**). This spatial separation was mainly influenced by variations in chlorophyll a (0.4981), chlorophyll b (0.4456), carotenoids (0.4251), free amino acids (0.3485), and proline (0.3096). The position of samples from Darshcolacke and Alte Mühle to the loadings indicates that they have lower pigments, amino acids, and proline content. Meanwhile, PC2 differentiation was driven by soluble sugars (0.5415), free amino acids (0.4474), proteins (-0.4296), and proline (0.4164), to which samples from April Seevorgelände and Sechsmahdlacke, were positively correlated, indicating their higher content. In September, the spatial separation was less distinct, with most AM replicates clustering on the opposite side of PC1 compared to other sites, although this was less pronounced than in April (**Figure 1**).

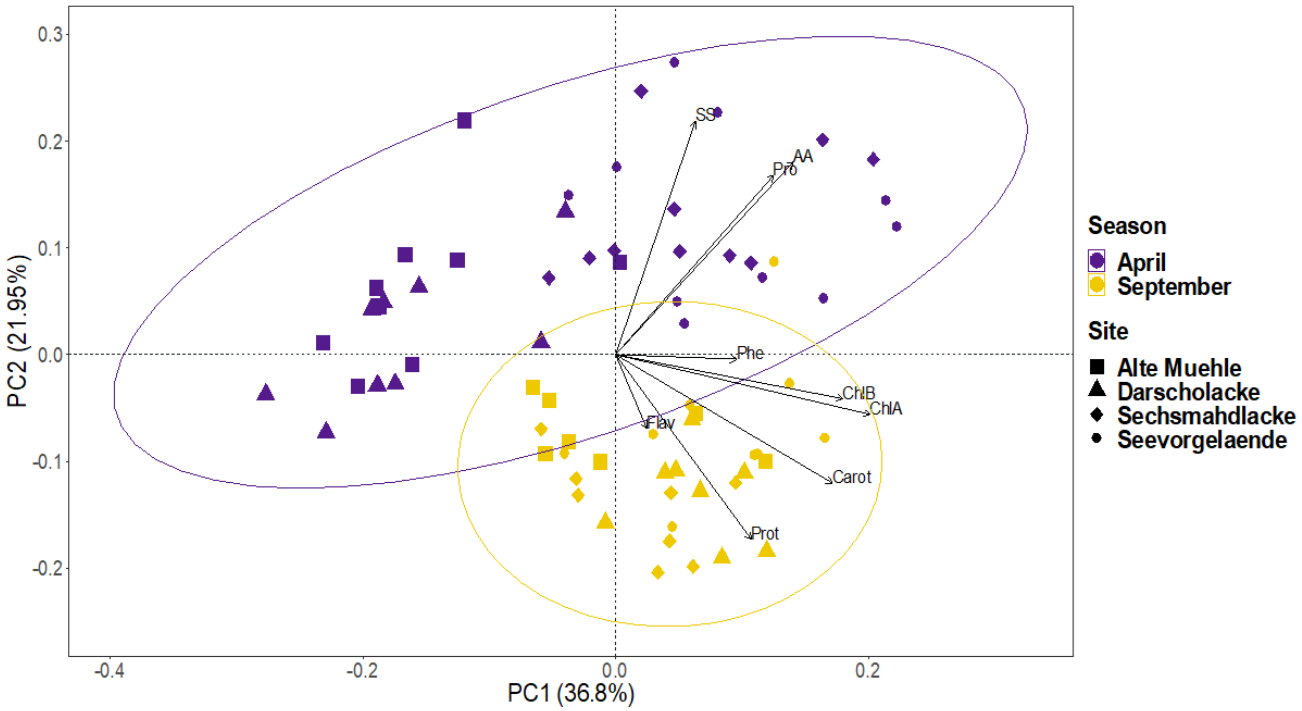


Figure 1: Principal component analysis shows seasonal and spatial separation in *Lotus tenuis* from Neusiedler See - Seewinkel. Loadings are depicted as arrows, with the direction and cosine angle of the slope of each arrow showing the correlation to principal components. The length of the arrows represents the strength of these correlations and the proportion of explained variance by the loading on each component. Seasons are represented by colors and sites are represented by shapes.

3.2.3 Comparative analysis of examined metabolites of *Lotus tenuis* plants

The following boxplots (**Figure 2-4**) depict spatial (**I**) and seasonal (**II**) changes across all 8 metabolites and total protein content (**a-i**). The results in the boxplot represent each group distribution, and significance letters (compact letter display) are assigned based on one-way ANOVA following Tukey HSD posthoc analysis.

3.2.3.1 Photosynthetic pigments content (Figure 2)

The analysis revealed significant spatial and seasonal differences in pigment concentrations (**Figure 2**). In April, there were more pronounced spatial differences in all pigment concentrations compared to September. Samples from Alte Mühle (AM) and Darscholacke (DL) in April differed the most, as indicated by the PCA (**Figure 1**). Specifically, chlorophyll a and carotenoid levels in April were significantly lower in samples from AM and DL compared to Sechsmahdlacke (SML) and Seevorgelände (SV) ($p < 0.05$) (**Figure 2 I-a,c**). Chlorophyll b content was also lower in April across AM and DL samples compared to SV and SML, significantly only to SV ($p < 0.05$) (**Figure 2 I-b**).

In September, SV samples maintained the highest pigment concentrations, while AM samples had the lowest. However, significant differences between AM and SV were found only for chlorophyll a and carotenoid levels ($p < 0.05$) (**Figure 2 I-a,c**). The content of chlorophyll b changed the least, and samples from all sites had relatively similar content, with the lowest at AM (9.32 ± 1.5 µg) and highest at SV (11.03 ± 0.75 µg). Seasonally, in chlorophyll a and b, all samples from April increased to September; in the content of carotenoids, only the SV sampled showed a decrease. AM and DL samples showed a 1.7-fold increase in chlorophyll a (**Figure 2II-a**), a 1.4- to 1.8-fold increase in chlorophyll b, respectively (**Figure 2 II-b**), and a 1.9-fold increase in carotenoids, with SV samples showing a 1.4-fold increase in carotenoids (**Figure 2 II-c**) ($p < 0.05$).

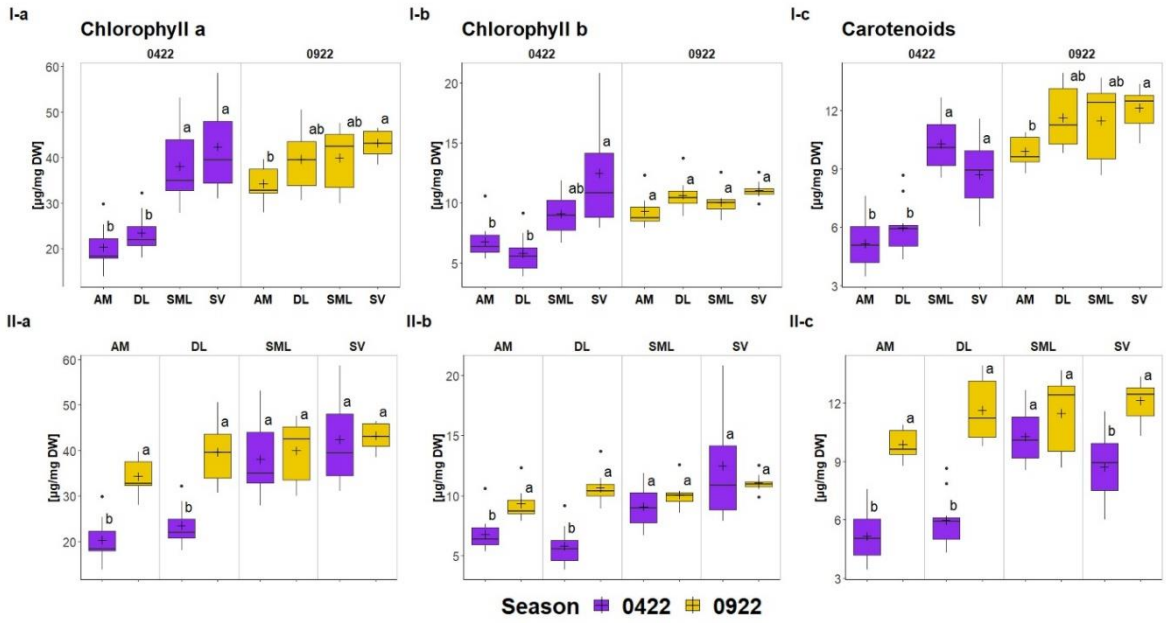


Figure 2: The boxplots illustrate pigment concentrations (chlorophyll a, chlorophyll b, and carotenoids) by season and site. The x-axis shows either sampling sites or seasons, with the y-axis representing concentrations per mg of dry weight (DW). A solid line indicates the median and the mean values by a "+." Letters indicate significant differences between groups; same letter - no significant difference ($p > 0.05$); different letters - significant differences between groups ($p < 0.05$); multiple letters suggest similarities with other groups; *0422 = April; 0922 = September; AM = Alte Mühle, DL = Darscholacke, SML = Sechsmahdlacke, SV = Seevorgelände*

3.2.3.2 Total phenols, flavonoids, and soluble sugars content (Figure 3)

Phenol concentrations revealed the most minor variability across all analyzed metabolites and total proteins. Hence, no significant seasonal or spatial differences in phenol concentration were found (**Figure 3 I-, II-d**). Samples from AM and DL behaved similarly and had, on average, lower content in April, whereas samples from SV and SML had lower content in September (Figure 3 II-d). Particularly in September, AM and DL samples had higher phenol content than SV and SML samples (**Figure 3 I-d**). Total flavonoid content (**Figure 3 e**) was across all samples the highest in DL from April and

September, both significantly than all other sites ($p < 0.05$) (**Figure 3 I-e**). Seasonally (**Figure 3 II-e**) replicates from AM, SML, and SV increased in flavonoid content from April to September, but significantly only AM by 1.4-fold and SV by 1.1-fold ($p < 0.05$) (**Figure 3 II-e**). Samples from DL had higher flavonoid content in April, but not significantly. The concentration of soluble sugars (**Figure 3 f**) spatially did not change significantly in any season (**Figure 3 I-f**). In September, the highest concentration was observed in samples from AM, followed by SV and DL, and the lowest concentration was observed in SML samples. Seasonally, as suggested in PCA (**Figure 1**), all samples in April showed, on average, higher concentrations of soluble sugars than in September, significantly only from SML by 1.5-fold and SV by 1.3-fold ($p < 0.05$) (**Figure 3 II-f**).

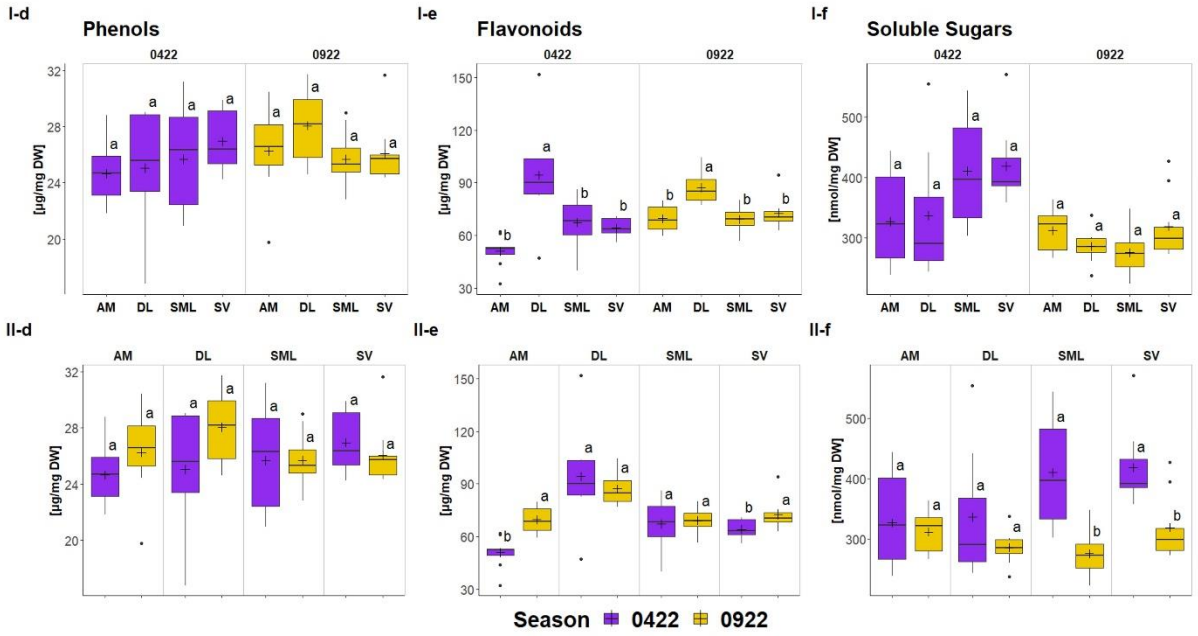


Figure 3: The boxplots illustrate phenols, flavonoids, and soluble sugars concentrations by season and site. The x-axis shows either sampling sites or seasons, with the y-axis representing concentrations per mg of dry weight (DW). A solid line indicates the median and the mean values by a "+." Letters indicate significant differences between groups; same letter - no significant difference ($p > 0.05$); different letters - significant differences between groups ($p < 0.05$); multiple letters suggest similarities with other groups; *0422 = April; 0922 = September; AM = Alte Mühle, DL = Darscholacke, SML = Sechsmahdlacke, SV = Seevorgelände*

3.2.3.3 Total free amino acids, proline, and protein content (Figure 4)

The concentration of free amino acids and proline was in April the lowest in AM and DL samples, remaining significantly lower than in SML and SV samples (**Figure 4 I-g,h**) ($p < 0.05$). Additionally, proline concentration was found to be the highest in samples from SML in April, also significantly higher than from SV ($p < 0.05$) (**Figure 4 I-h**). Total protein content was the lowest in samples from DL in April, also significant to all other groups ($p < 0.05$) (**Figure 4 I-i**). *L. tenuis* samples from September did not reveal any significant differences across free amino acids, proline, and total protein content, where amino acids were the highest in SV, proline, and proteins in DL samples. (**Figure 4 I-g, h, i**). Seasonally, free amino acids were found to decrease from April to September in samples from AM, SML, and SV, but significantly only in SML and SV ($p < 0.05$) (**Figure 4 II-g**), by 1.8- and 1.5-fold, respectively. Proline content, on average, decreased from April to September in samples from AM, SML, and SV, significantly for DL and SML by 1.8 and 2.8-fold, respectively ($p < 0.05$) (**Figure 4 II-h**). *L. tenuis* from DL revealed a significant increase in proline concentration by 1.8-fold from April to September ($p < 0.05$) (**Figure 4 II-h**). Total protein content seasonally changed the most, with a significant increase in protein concentration from April to September across all sites AM on average by ~3-fold ($p < 0.05$) (**Figure 4 II-i**).

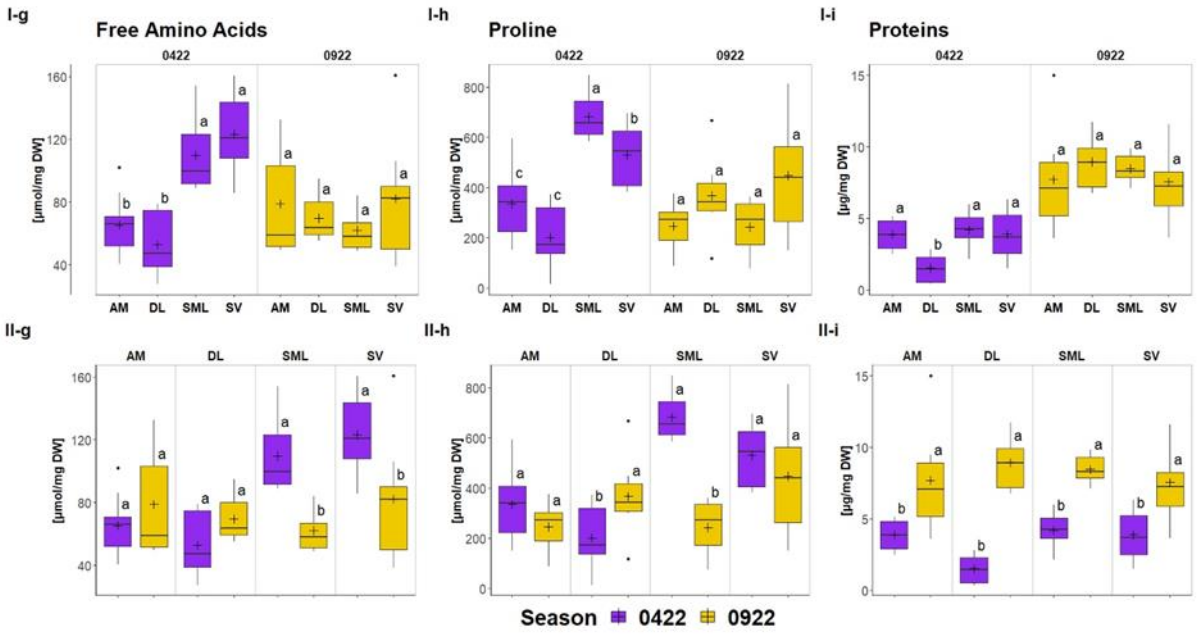


Figure 4: The boxplots illustrate free amino acids, proline, and total protein concentrations by season and site. The x-axis shows either sampling sites or seasons, with the y-axis representing concentrations per mg of dry weight (DW). A solid line indicates the median and the mean values by a "+." Letters indicate significant differences between groups; same letter - no significant difference ($p > 0.05$); different letters - significant differences between groups ($p < 0.05$); multiple letters suggest similarities with other groups; *0422 = April; 0922 = September; AM = Alte Mühle, DL = Darscholacke, SML = Sechsmahdlacke, SV = Seevorgelände*

3.2.4 Overall metabolites profile of *Lepidium cartilagineum*

Principal component analysis (**Figure 5**) represents 71.21% of the total variance partitioned between the first two axes (PC1 = 49.34%, PC2 = 21.85%). Overall, the most pronounced difference was observed at PC1, where samples from Alte Mühle in April separated from the rest, while replicates from September and Seevorgelände across both seasons clustered together (**Figure 5**). PC1 separation was influenced mostly by differences in concentrations of chlorophyll a (0.4318), b (0.4406), carotenoids (0.4436), phenols (0.3976), less by proteins (0.3157) and soluble sugars (-0.3049). This indicates a lower content of pigments in samples from Alte Mühle and higher soluble sugars relative to the rest of the replicates. Samples from Seevorgelände also revealed a slight differentiation on PC1; samples from September seem to have higher contents of pigments, phenols, carotenoids, and proteins based on their position according to loadings (metabolites). PC2 was slightly dividing Alte Mühle samples and Seevorgelände in both seasons. It was influenced mostly by differences in free amino acids (0.6645), proline (0.6698), and flavonoids (-0.2692); samples from September seem to differentiate less than in April (**Figure 5**).

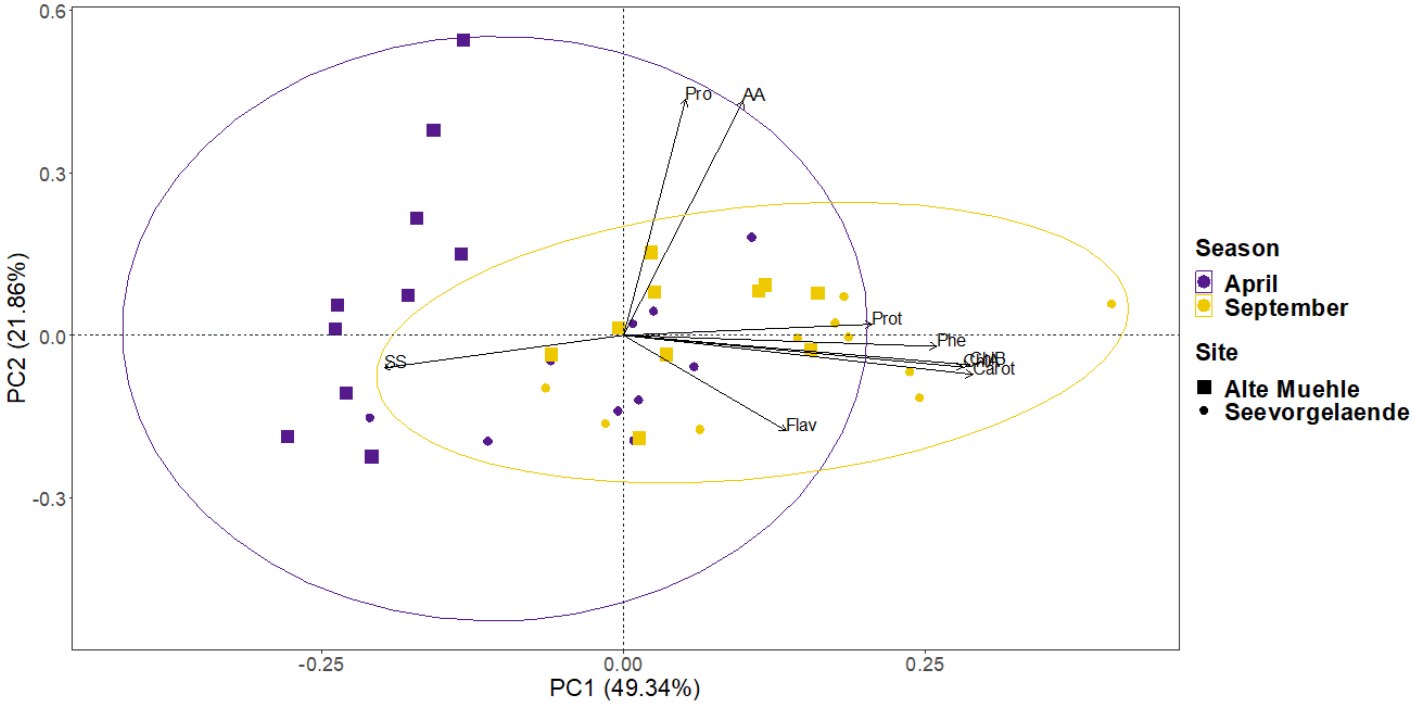


Figure 5: Principal component analysis shows seasonal and spatial separation in *Lepidium cartilagineum* from Neusiedler See - Seewinkel. Loadings are depicted as arrows, with the direction and cosine angle of the slope of each arrow showing the correlation to principal components. The length of the arrows represents the strength of these correlations and the proportion of explained variance by the loading on each component. Seasons are represented by colors and sites are represented by shapes.

3.2.5 Comparative analysis of examined metabolites: *Lepidium cartilagineum*

The following boxplots (**Figure 6-8**) depict spatial (**I**) and seasonal (**II**) changes across all 8 metabolites and total protein content (**a-i**). The results in the boxplot represent each group distribution, and significance letters (compact letter display) are assigned based on one-way ANOVA following Tukey HSD posthoc analysis.

3.2.5.1 Photosynthetic pigments content (Figure 6)

In April, Alte Mühle (AM) samples exhibited the lowest overall concentrations of photosynthetic pigments, which is also reflected in the PCA results (**Figure 5**). These concentrations were significantly lower than those observed at Seevorgelände in April (SV) ($p < 0.05$) (**Figure 6 I-a, b, c**). In September, AM samples still contained lower levels of photosynthetic pigments than SV; the difference was statistically significant only in carotenoid content ($p < 0.05$) (**Figure 6 I-c**). Seasonal analysis revealed that AM samples in April had significantly lower photosynthetic pigments content compared to September, lower by 1.7-fold in chlorophyll a, a 1.6-fold in chlorophyll b, and a 1.8-fold in carotenoids ($p < 0.05$) (**Figure 6 II-a,b,c**). Similarly, samples from SV also showed lower pigment levels in April compared to September, significantly lower by 1.3-fold in chlorophyll b and 1.2-fold in carotenoids (**Figure 6 II-b,c**) ($p < 0.05$).

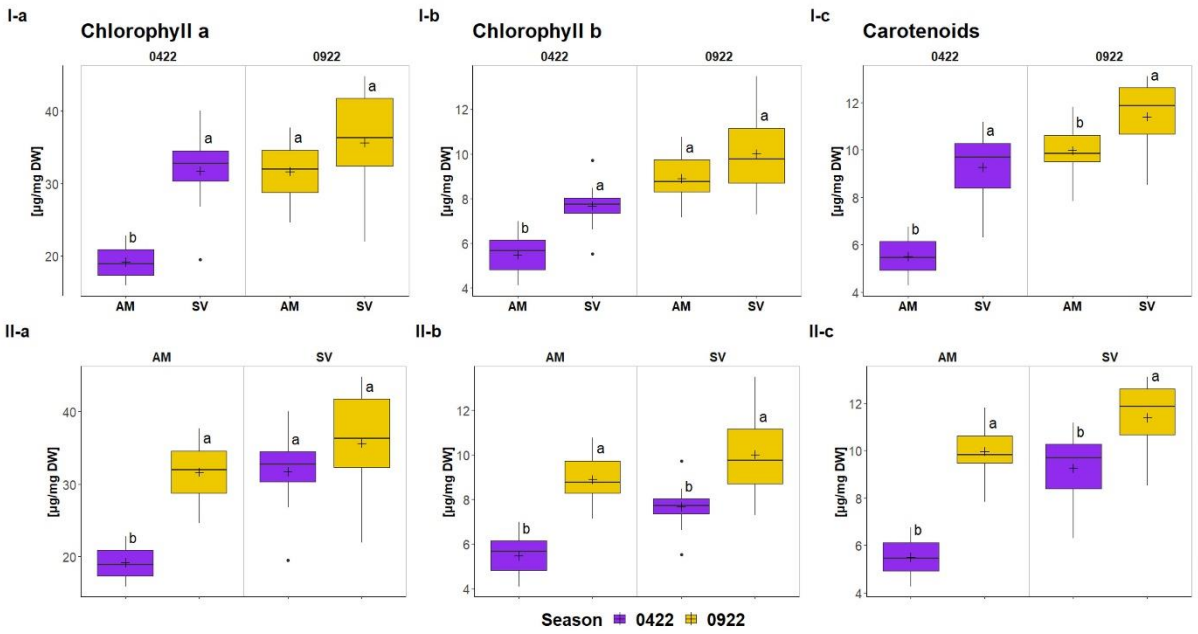


Figure 6: The boxplots illustrate pigment concentrations (chlorophyll a, chlorophyll b, and carotenoids) by season and site. The x-axis shows either sampling sites or seasons, with the y-axis representing concentrations per mg of dry weight (DW). A solid line indicates the median and the mean values by a "+." Letters indicate significant differences between groups; same letter - no significant difference ($p > 0.05$); different letters - significant

differences between groups ($p < 0.05$); multiple letters suggest similarities with other groups; *0422 = April; 0922 = September; AM = Alte Mühle, SV = Seevorgelände*

3.2.5.2 Total phenols, flavonoids, and soluble sugars content (Figure 7)

The analysis of phenols (**Figure 7 I-d**) and flavonoids (Figure 7 I-e) revealed a consistent spatial pattern across both seasons, with higher concentrations in samples from Seevorgelände (SV) compared to Alte Mühle (AM). However, these differences were statistically significant only in April for both metabolites ($p < 0.05$) (**Figure 7 I-d, e**). In contrast, soluble sugars exhibited an opposite trend, with lower concentrations in SV samples across both seasons and higher spatial differences in September, in AM samples significantly lower than in SV ($p < 0.05$) (**Figure 7 I-f**). Seasonal changes in phenol content were observed in samples from both sites (AM and SV), with a significant increase of approximately 1.2-fold from April to September (**Figure 7 II-d**). Flavonoid content, on the other hand, decreased from April to September in samples from both sites (**Figure 7 II-e**). Soluble sugars showed a significant decline from April to September, with a 1.03-fold decrease at AM and a 1.3-fold decrease at SV ($p < 0.05$) (**Figure 7 II-f**).

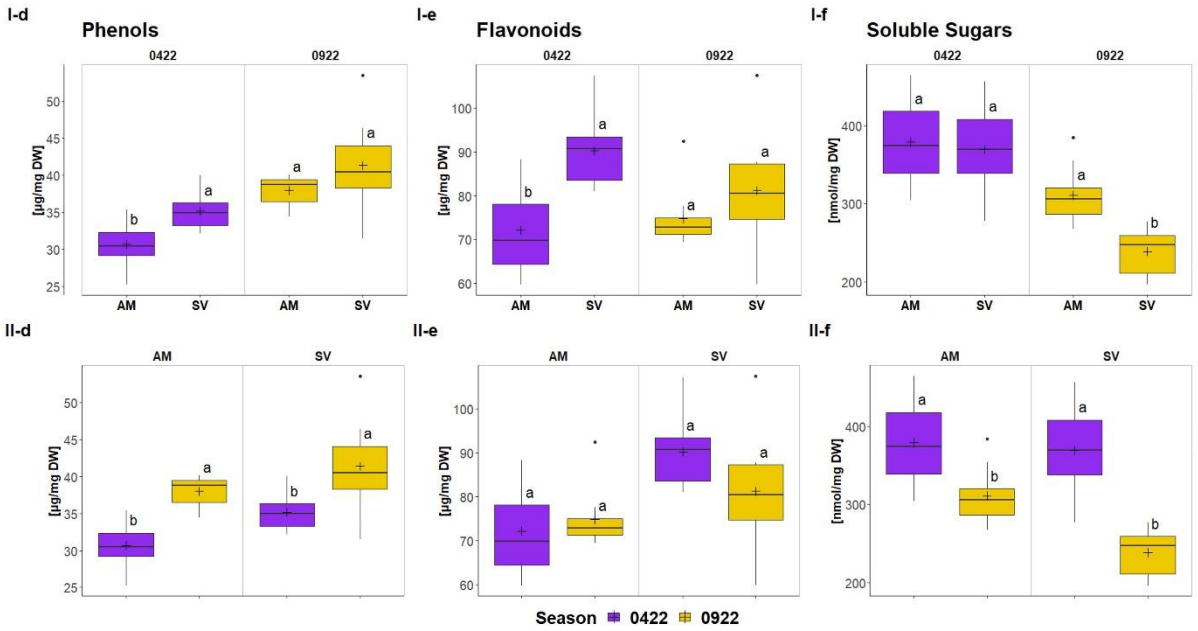


Figure 7: The boxplots illustrate pigment concentrations of phenols, flavonoids, and soluble sugars by season and site. The x-axis shows either sampling sites or seasons, with the y-axis representing concentrations per mg of dry weight (DW). A solid line indicates the median and the mean values by a "+." Letters indicate significant differences between groups; same letter - no significant difference ($p > 0.05$); different letters - significant differences between groups ($p < 0.05$); multiple letters suggest similarities with other groups; *0422 = April; 0922 = September; AM = Alte Mühle, SV = Seevorgelände*

3.2.5.3 Total free amino acids, proline, and protein content (Figure 8)

Overall, there were no significant spatial differences in amino acids and proline content across both seasons (**Figure 8 g, h**), with the concentrations of both metabolites being higher in samples from Alte Mühle (AM). Spatial variations in protein content were minimal, with both sites exhibiting very similar concentrations across the seasons (**Figure 8 I-i**). Regarding seasonal changes (**Figure 8 II-g, h**), amino acid content was higher in samples from September than in April, while proline content exhibited the opposite trend and was found in higher content in April samples. Protein content displayed a seasonal trend, with significantly higher concentrations in September samples at both sites ($p < 0.05$), averaging a seven-fold increase (**Figure 8 II-i**) (suggested in PCA in **Figure 5**).

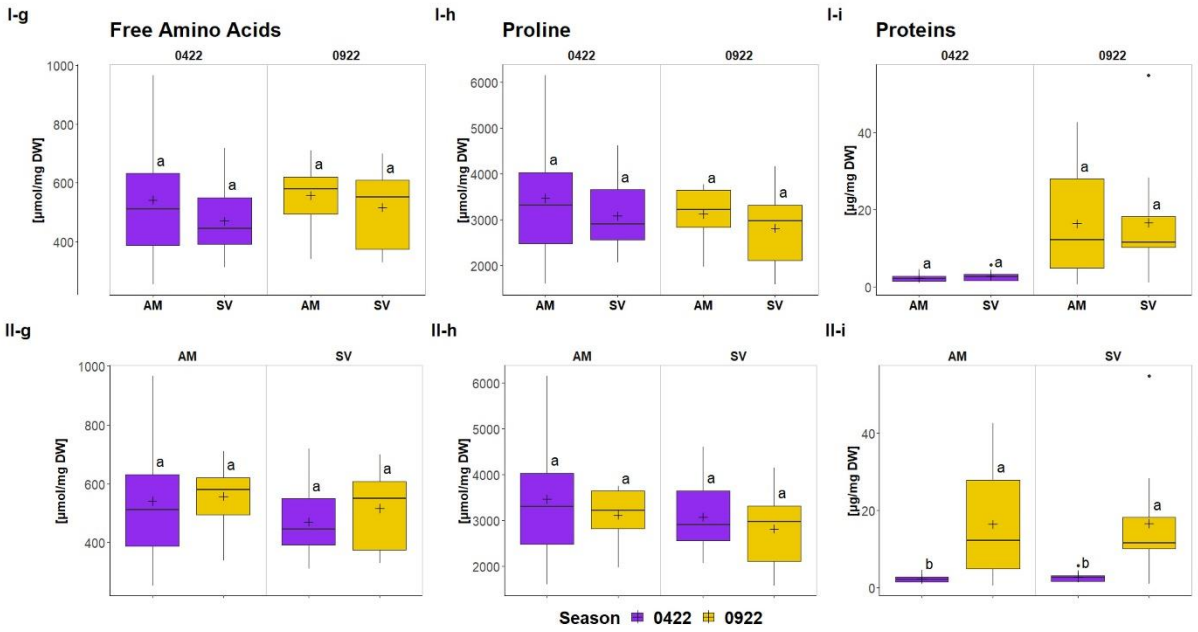


Figure 8: The boxplots illustrate free amino acids, proline, and total protein concentrations by season and site. The x-axis shows either sampling sites or seasons, with the y-axis representing concentrations per mg of dry weight (DW). A solid line indicates the median and the mean values by a "+." Letters indicate significant differences between groups; same letter - no significant difference ($p > 0.05$); different letters - significant differences between groups ($p < 0.05$); multiple letters suggest similarities with other groups; *0422 = April; 0922 = September; AM = Alte Mühle, DL = Darscholacke, SML = Sechsmahdlacke, SV = Seevorgelände*

3.3 Integrative metabolite and soil properties analysis – Neusiedler See – Seewinkel

The impact of analyzed soil properties on metabolite and total protein content changes was further examined using redundancy analysis (RDA). To assess the influence of soil properties on metabolites, separate RDA was performed for wild *Lotus tenuis* and *Lepidium cartilagineum* plants (from Neusiedler See-Seewinkel). For analysis, only soil properties that did not correlate to each other and still contributed to the explained variance were chosen (*correlations are in **Table S3** and **S7***). The RDA was

followed by significance testing of the model and the effects of soil parameters on metabolites and the axes using permutation ANOVA (***Table S1, S5***). Given the differences in metabolites and total protein content between the two species and the variations in soil profiles across sampling sites, a distinct set of soil properties was selected to correlate to metabolites and total protein content for each plant. The results are represented in the ordnation plots (**Figure 9,10**).

3.3.1 *Lotus tenuis* soil properties to metabolite correlation using redundancy analysis

Soil properties selected for analysis included soil water content, concentrations of $Mg^{2}+$, CO_3^{2-}, and Na^+ ions, conductivity, and pH. The RDA model explained 52.11% of the variation in metabolite data (R^2 adjusted = 35.20%), which was partitioned across six RDA axes corresponding to the selected soil properties. The first two axes accounted for 32.33% of the variance (RDA1 = 19.4%; RDA2 = 12.93%) (Figure 9). PERMANOVA (1000 permutations) confirmed the significance of the entire model ($p < 0.001$), with axes 1-3 being significant (axis 1: $p < 0.01$; axes 2 and 3: $p < 0.05$).

Among the explanatory variables, pH had a significant effect ($p < 0.01$), strongly influencing RDA1 (0.5384) and moderately affecting RDA2 (0.2374). pH was positively associated with protein and pigment concentrations and negatively correlated with sugars, flavonoids, and amino acids (**Figure 9**). Conductivity also showed significant effects ($p < 0.001$), negatively influencing RDA1 (-0.5897) and correlating weakly with RDA2 (0.4371). Its effects were opposite to pH's, positively impacting flavonoids, sugars, and amino acids, while negatively affecting proteins and pigments. Both conductivity and pH weakly influenced proline.

Of the ions, Mg^{2+} ($p < 0.01$) and Na^+ ($p < 0.05$) were significant. Magnesium predominantly influenced RDA1 (-0.6338) and had a weaker positive influence on RDA2 (0.255), with effects similar to conductivity. Sodium negatively influenced RDA1 (-0.3659) and had a minimal effect on RDA2 (-0.01426), showing similar, but weaker, effects on metabolites as conductivity. Neither soil water content (SWC) nor CO_3^{2-} showed significant effects on metabolite concentrations, and both had weak influences on the RDA axes. Differences in any analyzed soil parameters did not seem to change phenol content and exhibited no significant seasonal or spatial variation (**Figures 3 I, II-d**, and **9**).

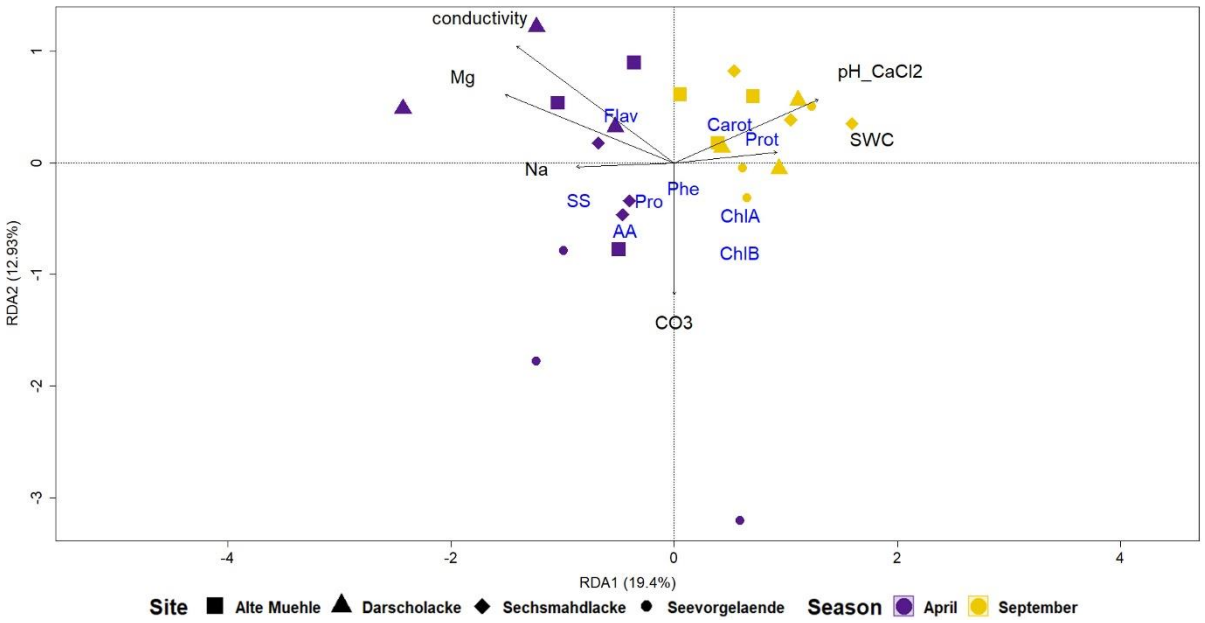


Figure 9: The RDA ordination plot (scaling 2 method – correlation plot) shows the estimated relationship between metabolites and soil properties across all sites and seasons. The relationship to each site and season is depicted by proximity. The arrows represent soil properties. The cosine angle between variables depicts their correlation relationship, and the length depicts the correlation strength of each variable to the ordination axes. Metabolite concentrations are depicted in blue. The cosine angle of metabolites and their position to soil properties approximates their correlations.

3.3.2 *Lepidium cartilagineum* soil properties to metabolite correlation using redundancy analysis

The variables contributing to the explained variance and not correlated with each other were soil water content (SWC), conductivity, pH, Mg^{2+}, Cl^{-}, SO_4^{2-}. These soil properties accounted for 75.16% of the variation in metabolite content (R^2 adjusted = 45.34%). The first two axes of the RDA explained 62.61% of the variance (RDA1 = 47.12%, RDA2 = 15.49%) (**Figure 10**). The overall model was significant ($p < 0.05$), with only axis 1 being significant, suggesting a dominant factor or a combination of correlated factors driving the primary pattern in the metabolite data.

Among the six explanatory variables, three had significant effects. pH ($CaCl_2$) had the most substantial influence on RDA1 (0.5759, $p < 0.05$), primarily affecting chlorophyll a, b, carotenoids, proteins, and phenols while negatively impacting soluble sugars. Magnesium (Mg^{2+}) was significant ($p < 0.01$) and primarily influenced RDA2 (0.5296), correlating with proline and amino acid content (**Figure 10**), which did not reveal any significant differences across sites or seasons (**Figure 8-g,h**). Chloride also had a significant effect ($p < 0.05$), positively influencing soluble sugars and negatively impacting chlorophyll a and b, proteins, phenols, and flavonoids on RDA1 (-0.4789). Soil water content had the

weakest, non-significant effect, while conductivity had weak, non-significant influences on RDA1 and RDA2, respectively, not revealing significant differences across samples (**Table 3**). Sulfate was evenly influencing sugars and amino acids in April, but not significantly.

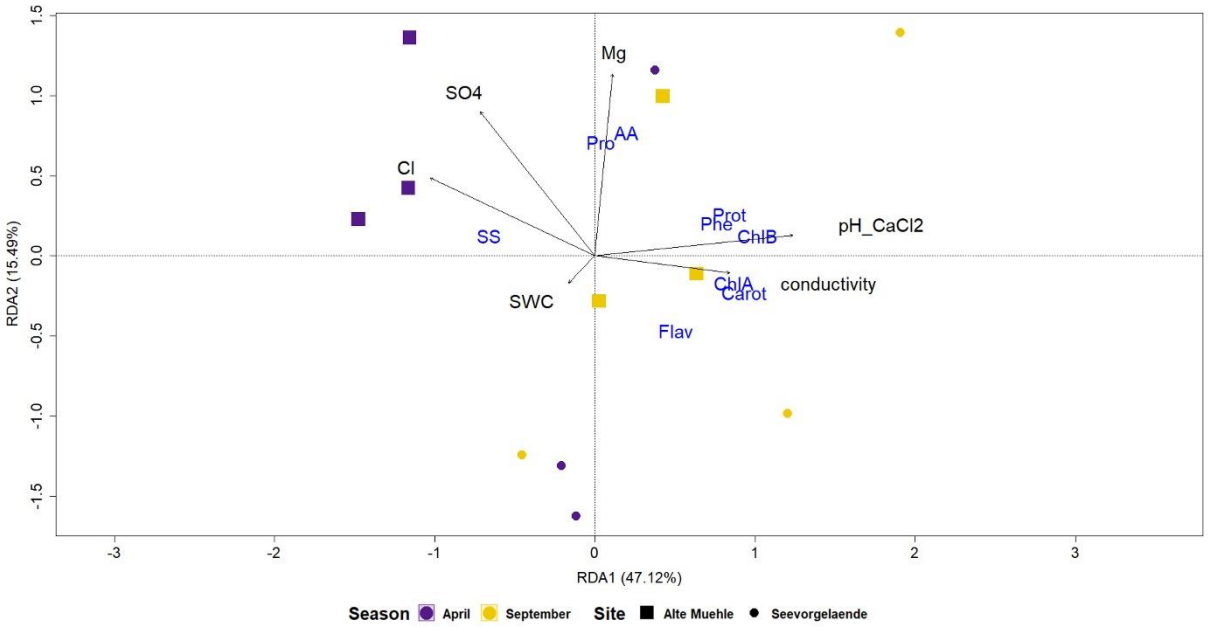


Figure 10: The RDA ordination plot (scaling 2 method – correlation plot) shows the estimated relationship between metabolites and soil properties across all sites and seasons. The relationship to each site and season is depicted by proximity. The arrows represent soil properties. The cosine angle between variables depicts their correlation relationship, and the length depicts the correlation strength of each variable to the ordination axes. Metabolite concentrations are depicted in blue. The cosine angle of metabolites and their position to soil properties approximates their correlations.

3.4 Comparison of metabolite profiles of wild plants *Lotus tenuis* and *Lepidium cartilagineum* with controlled growth-chamber plants and to model species

This analysis aims to evaluate how the metabolic state of wild plants from the Neusiedler See-Seewinkel region compares to those under optimal growth chamber conditions. Additionally, the investigation aims to elucidate the inherent metabolic adaptations of halophytes relative to non-halophytic controls within the same taxonomic family when not subjected to non-saline environments. As a control for both wild plants, the same species, *L. tenuis* and *L. cartilagineum*, were cultivated. Since the status and efficiency of *Lotus tenuis* symbiotic nitrogen fixation from various sites were unknown, we also compared the metabolite concentrations to Rhizobium inoculated and non-inoculated plants. Furthermore, an additional threshold metabolite concentration of well-characterized species from the same family, *Lotus japonicus* and *Lepidium sativum,* growing in the same conditions and treated as Lotus tenuis and Lepidium cartilagineum was conducted. The data were analyzed as one dataset, and the concentrations of metabolites and total proteins extracted from wild plants and controls were

compared to all control species of the same family (***Table S14 , S21***). First, a Principal Component Analysis (PCA) was conducted to gain insight into the variation among the compared groups (**Figure 11, 14**). Subsequently, a one-way ANOVA with Dunnett's multiple comparison tests was performed to identify significant differences between the controls and wild plants controls for each metabolite and total protein content separately. Data are represented in boxplots (**Figure 12,13,15,16**).

3.4.1 *Lotus tenuis* wild plants and controls metabolite profile

Figure 11(**I**) illustrates that 64.40% of the variance among the two control species of Lotus under nitrogen-fixing (N-fix) and nitrogen-fed (N-fed) conditions, as well as the wild plants, is explained by PC1 with 46.68%. On PC1, wild *Lotus tenuis* (LT) plants from Darscholacke (DL) and Alte Mühle (AM) in April cluster separately from the other wild samples, aligning more closely with the control plants, particularly nitrogen-fed *L. tenui*s and both *L. japonicus* under treatment conditions (**Figure 11 I**). This separation on PC1 is primarily driven by differences in chlorophyll a (0.4224), chlorophyll b (0.3830), carotenoids (0.3915), and free amino acids (0.3250), which are on PC1 indicated to be in lower content in AM, DL April, LJ and LT N-fed (**Figure 11 I**). The *L. tenuis* samples from April, in the PC2 (17.7%), vary more to all controls; this is largely influenced by differences in soluble sugars (0.5136), proteins (-0.4483), carotenoids (-0.3375) and free amino acids (0.3168) content (**Figure 11 I**). The controls and the most - nitrogen-fixing *L. tenuis* exhibit similarities to the wild samples from September on PC2. (**Figure 11 I**).

Figure 11 (**II**) explains 74.28% of the total variation in metabolite concentrations. Separation along PC1 (56.52%) differentiates between species and more N-fix treatments of both plants, while N-fed treatments show greater variation along PC2 (17.76%). The differences along PC1 are uniformly influenced by all metabolites (ranging from -0.4032 to -0.3338), except for proline and proteins, which have a lesser impact (-0.1920 and -0.2443, respectively), indicating that different species differ in those metabolites, regardless of treatment (**Figure 11 II**). PC2 is mainly driven by proline concentrations (-0.5558), along with carotenoids and chlorophyll a (0.4342 and 0.3917, respectively).

The differentiation between wild and control plants of *L. tenuis* (**Figure 11 III**), without considering *L.japonicus* (**Figure 11 I**), remains pronounced on PC1 (39.76%), separating wild plants (April and September) from AM and DL in April, and controls—especially N-fed treatment. PC1 remains primarily influenced by chlorophyll a, chlorophyll b, and carotenoids (0.4570, 0.4127, and 0.4049, respectively) and free amino acids (0.3465). PC2 (20.47%) remains to reflect the seasonal separation between April and September wild plants, with the controls aligning more closely with September samples, particularly those under the N-fix treatment, driven by soluble sugar content (0.5452), proteins (-0.3667) and phenols (0.3506) (**Figure 11 III**).

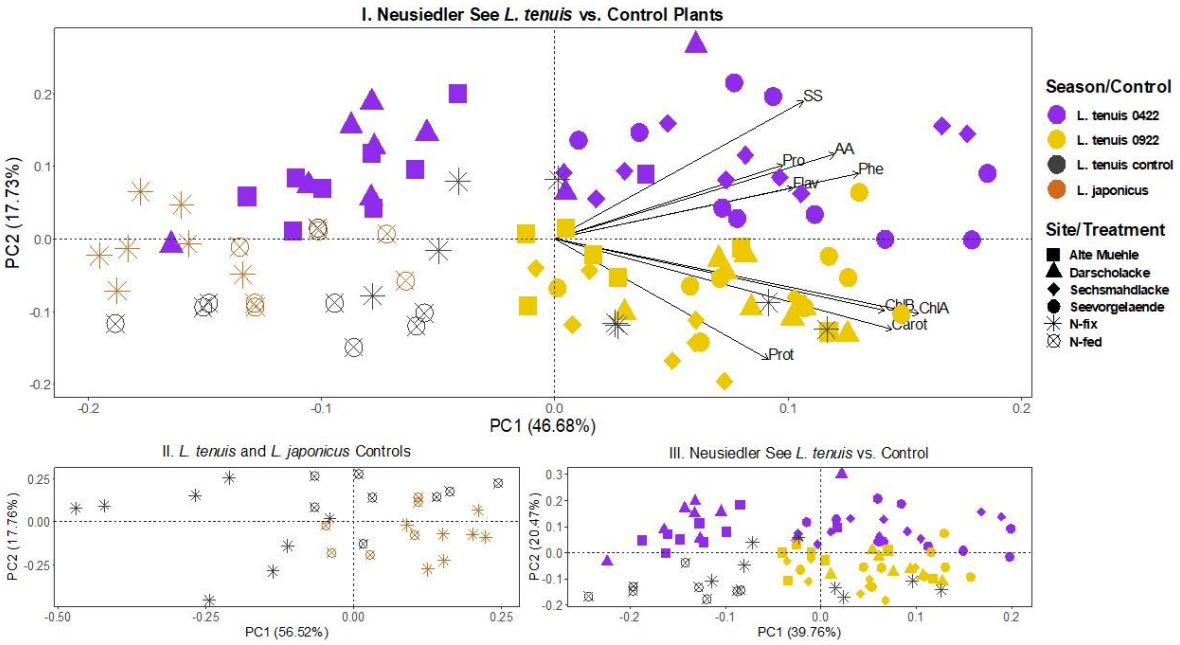


Figure 11: Principal Component Analysis (PCA) comparing *Lotus tenuis* from the Neusiedler See with control species (*L. tenuis* and *L. japonicus*) under two regimes: nitrogen-fixing (N-fix) and nitrogen-fed (N-fed). The large graph (**I**) captures the overall variation, showing how wild *L. tenuis* across all sites (shapes) and seasons (colors) compares to the control species (color) in both regimes (N-fix and N-fed; indicated by shape). The smaller inset graphs: **II** provides a more detailed comparison between the control species alone; **III** focuses on the variation between *L. tenuis* from different sites and seasons and the control treatments. Before conducting the PCA, data for graphs II and III were separated into corresponding datasets; n = 6-10

3.4.3 Comparative analysis of metabolites in control plants: *Lotus japonicus* and *Lotus tenuis*

In general, nitrogen-fixing (N-fix) *Lotus tenuis* (LT) exhibited the highest concentration across all metabolites and total protein content (**Figure 12**). *Lotus japonicus* (LJ) N-fix had the lowest concentration across all pigments (**Figure 12 a-c**). The most pronounced and significant differences ($p < 0.05$) were observed between LT N-fix and LJ N-fix, particularly across 5 out of the 9 metabolites compared (**Figure 12 a, b, c, g, h**).

The content of chlorophyll a and carotenoids showed higher variation across samples than chlorophyll b. In general, was, chlorophyll b significantly higher in Lotus tenuis N-fix, compared to L. japonicus in both regimes and *L. tenuis* N-fed ($p < 0.05$) (**Figure 12 b**). *L. tenuis* N-fix, with the highest content of all pigments, also had a substantially higher content of chlorophyll a and carotenoids than both L. japonicus treatments ($p < 0.05$) (**Figure 12 a,c**). *Lotus tenuis* N-fed exhibited the second highest content of pigments and contained more chlorophyll a and b than L. japonicus N-fix ($p < 0.05$) (**Figure 12 a,c**). Oppositely to *L. tenuis* plants, *L. japonicus* N-fed exhibited higher content of all pigments than N-fix

L. japonicus, significantly higher than N-fix ($p < 0.05$). N-fed treatments had similar content of both concentrations.

Phenols were found to be significantly higher in *L. tenuis* N-fix, than in both N-fed plants ($p < 0.05$); LT N-fed and *L. japonicus* in both treatments had similar content of phenols (**Figure 12 d**). In flavonoids, content the biggest differences were found between plants across opposed regiments, *L. tenuis* N-fix had significantly higher content than *L. japonicus* N-fed ($p < 0.05$) (**Figure 12 e**). Concentrations of soluble sugars did not change significantly between compared groups; the highest differences were between *Lotus tenuis* regiments, where N-fed had the lowest content of soluble sugars (**Figure 12 f**).

Free amino acids, proline, and protein content changed the most between *L. tenuis* opposite regiments and *L. japonicus* N-fix regiment (**Figure 12 g,h,i**). Free amino acids and proline had the same significant differences found. Significantly higher content in *L. tenuis* N-fix to N-fed and to LJ-Nfix ($p < 0.05$) (**Figure 12 g,h**). Control plants did not reveal any significant differences in protein content and had very similar concentrations across compared groups (**Figure 12 i**).

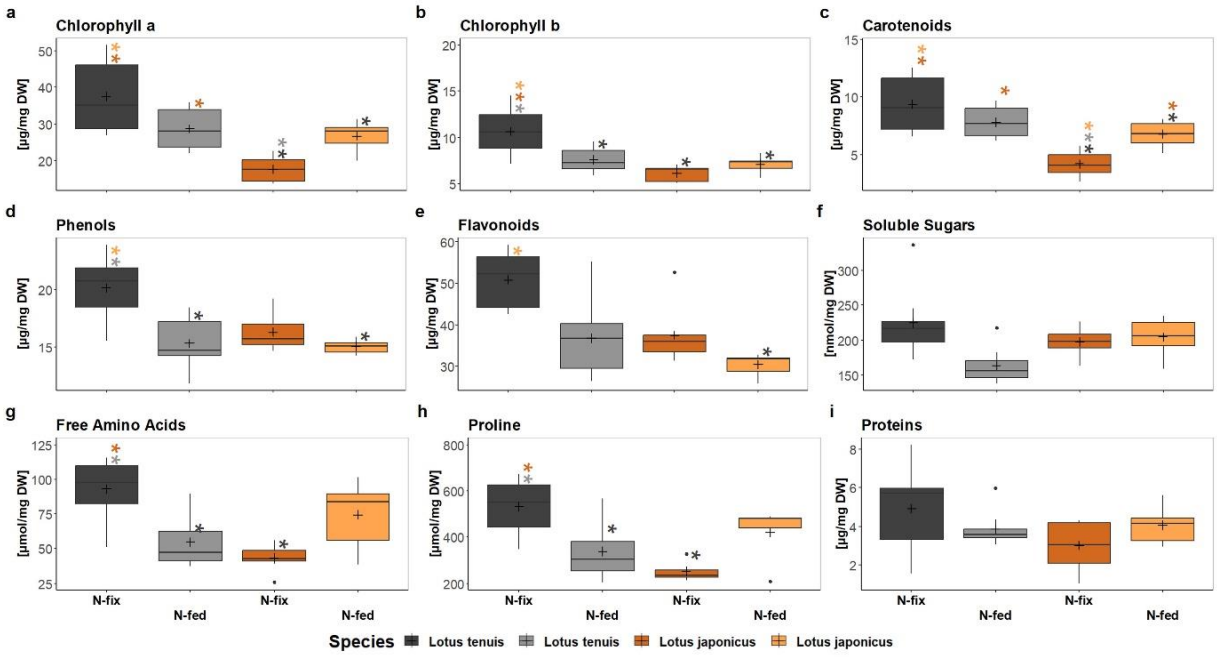


Figure 12: Boxplot representing metabolite concentrations in control plants*, Lotus tenuis,* and *Lotus japonicus,* across nitrogen regimes N-fix and N-fed. X-axis depicting the nitrogen regimes, N-fix – nitrogen fixing control, N-fed – nitrogen fed control (not inoculated). Y-axis depicting the concentration per mg of dry weight (DW). Error bars are the first and fourth quartiles. Mean values are represented by "+". Vertical stars stand for significant differences found by separate post-hoc - Dunnett-tests; the color coding stands for significant relation to *Lotus tenuis* N-fix (dark grey), N-fed (light grey); *Lotus japonicus* N-fix (dark brown), and N-fed (light brown); *p-value coding = $p < 0.05$ *, n = 6-8.*

3.4.4 Comparative analysis of metabolites of *Lotus tenuis* control and wild plants

Analysis of variance followed by the Dunnett test revealed significant differences across wild plants and controls of *Lotus tenuis* in all metabolites. Overall, in chlorophyll a, carotenoids, flavonoids, sugars, and proteins (**Figure 13 a, c, e, f, i)** the metabolite concentration in both N-fix and N-fed regiments did not differ significantly, as already visible in **Figure 12**. Wild plants of L. *tenuis*, concretely from Darscholacke in April (DL0422), remained significantly different from N-fix controls across all metabolites. However, the inoculation was carried out using soil originating from that site.

photosynthetic pigments (a, b, c)

Samples from Alte Mühle and Darscholacke in April (0422) had similar content as N-fed *Lotus tenuis* controls and significantly lower content than Nitrogen-fixing (N-fix) *Lotus tenuis* (LT) controls ($p < 0.05$) (**Figure 13 a-c**).

Oppositely, plants from SML and SV sites in April exhibited higher content of pigments, similar to N-fix LT and significantly higher chlorophyll a than N-fed LT controls ($p < 0.05$); SV plants also had higher chlorophyll b content and SML carotenoids content ($p < 0.05$) (**Figure 13 a,b,c**). All wild plants from September (0922) had similar content of pigments to N-fixing LT controls, whereas AM 0922 did not reveal any significant differences in the contents of all pigments to both control groups (**Figure 13 a-c**). Chlorophyll a and carotenoids were significantly higher in September plants from DL, SML, and SV ($p < 0.05$) compared to N-fed controls (**Figure 13 a,c**). Chlorophyll b remained significantly higher only in plants from DL and SV compared to N-fed LT ($p < 0.05$) (**Figure 13 b**). Besides, carotenoid content was in samples from DL and SV 0922 the highest, significantly higher than N-fixing LT control ($p < 0.05$) (**Figure 13 c**).

phenols, flavonoids, soluble sugars (d, e, f)

Phenols (**Figure 13 d**) concentrations across all wild *Lotus tenuis* sample groups were significantly higher than controls N-fix and N-fed ($p < 0.05$); N-fix also higher than N-fed ($p < 0.05$) (**Figure 13, 11 d**). Wild plants generally exhibited higher flavonoid content than nitrogen-fixing and nitrogen-fed controls, which had the lowest (**Figure 13 e**). Control groups and AM in April did not differ significantly and had very low flavonoid content. Samples from September across all sites had substantially higher flavonoid content than controls N-fix and N-fed ($p < 0.05$). In April, DL and SML followed the same trend, and SV showed significantly higher content than N-fed ($p < 0.05$) (**Figure 13 e**). The concentration of soluble sugars was the lowest in N-fed plants of *L. tenuis* and significantly lower than all wild plants controls in April and September ($p < 0.05$) (**Figure 13 f**). Nitrogen-fixing controls had a higher content of soluble sugars. However, they remained significantly lower than

replicates from all sites in April and lower than AM and SV replicates in September ($p < 0.05$) (**Figure 13 f**). Controls did not differ significantly.

free amino acids, proline, proteins (g, h, i)

Free amino acids and proline content (**Figure 13 g, h**) were significantly higher in April SML and SV samples compared to N-fed controls of *L. tenuis* ($p < 0.05$), where N-fed, AM and DL in April had overall the lowest content of both metabolites, N-fed and DL also significantly lower than N-fix controls ($p < 0.05$). Samples from September did not reveal any significant differences between N-fed and N-fix controls in free amino acids content, but overall, lower than N-fix and higher than N-fed (**Figure 13 g**). Nitrogen-fixing plants had also higher proline content than samples from AM and DL in April as well as AM and SML from September ($p < 0.05$) (**Figure 13 h**). The concentration of proteins (**Figure 13 i**) was significantly higher in samples from all sites in September compared to LT N-fix and N-fed. Additionally, DL exhibited the lowest protein content, significantly lower than LT N-fix control ($p < 0.05$) (**Figure 13 i**). Control groups were on average in similar range of protein concentrations and did not reveal any significant differences.

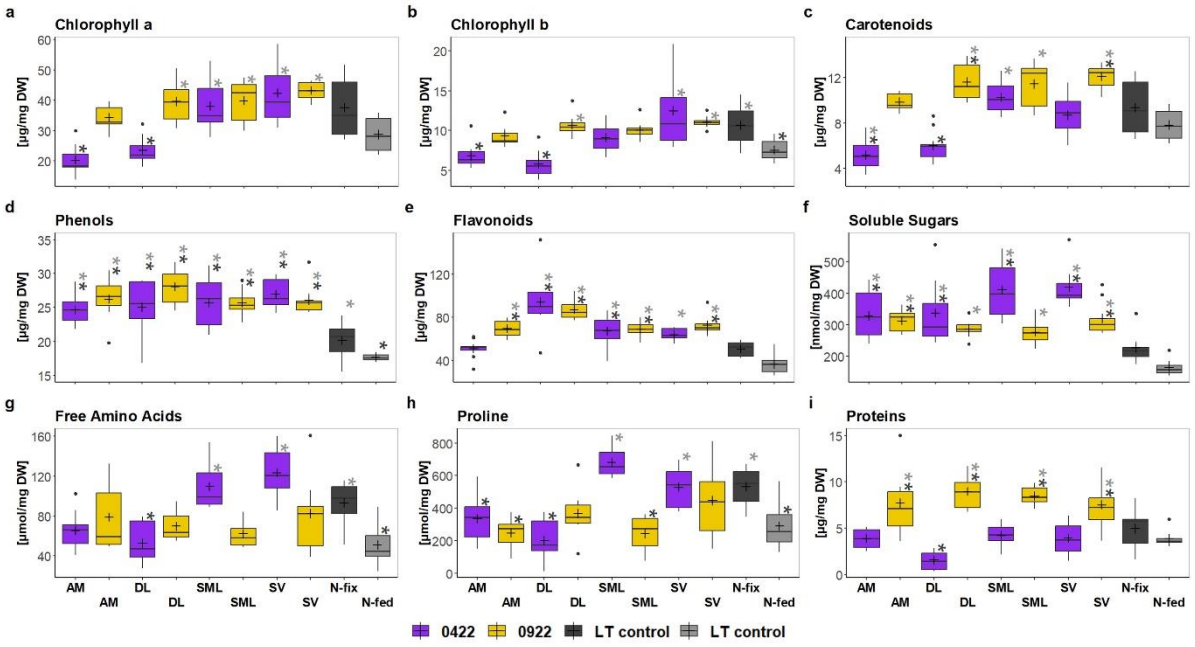


Figure 13: Boxplot representing metabolite concentrations across sites and seasons compared to controls Lotus tenuis N-fix and N-fed. X-axis depicts the site acronym (AM – Alte Mühle, DL – Darscholacke, SML – Sechsmahdlacke, SV – Seevorgelaende), and the numbers represent the 2 seasons (0422 – April, 0922 – September) LT – *Lotus tenuis*, N-fix – nitrogen fixing control, N-fed – nitrogen fed control (not inoculated). Y-axis depicting the concentration per mg of dry weight (DW). Error bars are the first and fourth quartiles. Mean values are represented by "+". Vertical stars stand for significant differences found by separate post-hoc - Dunnett-tests; the color coding stands for significance to N-fix (dark grey) or N-fed (light grey), p-value coding = $p < 0.05$ *; n = 7-10.

3.4.5 Comparative analysis of metabolites of *Lepidium cartilaginuem* control and wild plants

Figure 14 (**I**) illustrates 70.94% variance partitioned between 2 axes, PC1 (45.57) and PC2 (25.37%), and show differentiation of *Lepidium cartilagineum* species – wild and control, and model species *L. sativum.* The biggest differentiation is visible on PC1 between different species; both *L. cartilagineum* (wild and control) cluster on one side oppositely to *L. sativum*. This separation is driven mainly by differences in carotenoids (0.4440), chlorophyll b (0.3886), chlorophyll a (0.3835), free amino acids (0.3829), and proline (0.3659) contents (**Figure 14 I**). On PC2, the separation is visible for both controls, and wild plants, caused mostly by different content of soluble sugars (0.5811), phenols (0.3702), flavonoids (0.3317), chlorophyll a and b (0.3536, and 0.3664, respectively) (**Figure 14 I**). Figure 14 (II) represents the differentiation of control species. On PC1 (68.22%) is visible clustering of both species at opposite sites, influenced by differences in the content of chlorophyll a, b, carotenoids (0.3950, 0.3931, 0.3972, respectively), sugars (0.3860) and free amino acids (0.3962). There was no clustering along PC2 (14.33%) based on differences between species, but rather, some samples across both revealed higher variability in flavonoid content (-0.7397) (**Figure 14 II**). In **Figure 14** (**III**) is a closer image of wild *Lepidium cartilagineum* species across sites and seasons in regard to *L. cartilagineum* controls. The strongest separation was on PC1 (40.35%) between wild plants and controls, caused in differences in chlorophyll a (0.4819), b (0.4916), carotenoids (0.4711) and soluble sugars (-0.41) content. Some September replicates mostly from Seevorgelände were on PC1 similar to controls. On PC2 (21.68%), samples from Alte Mühle in April were also separated from other samples of both seasons, and were similar conent of phenols (0.6328) and flavonoids (0.606) as controls (**Figure 14 III**).

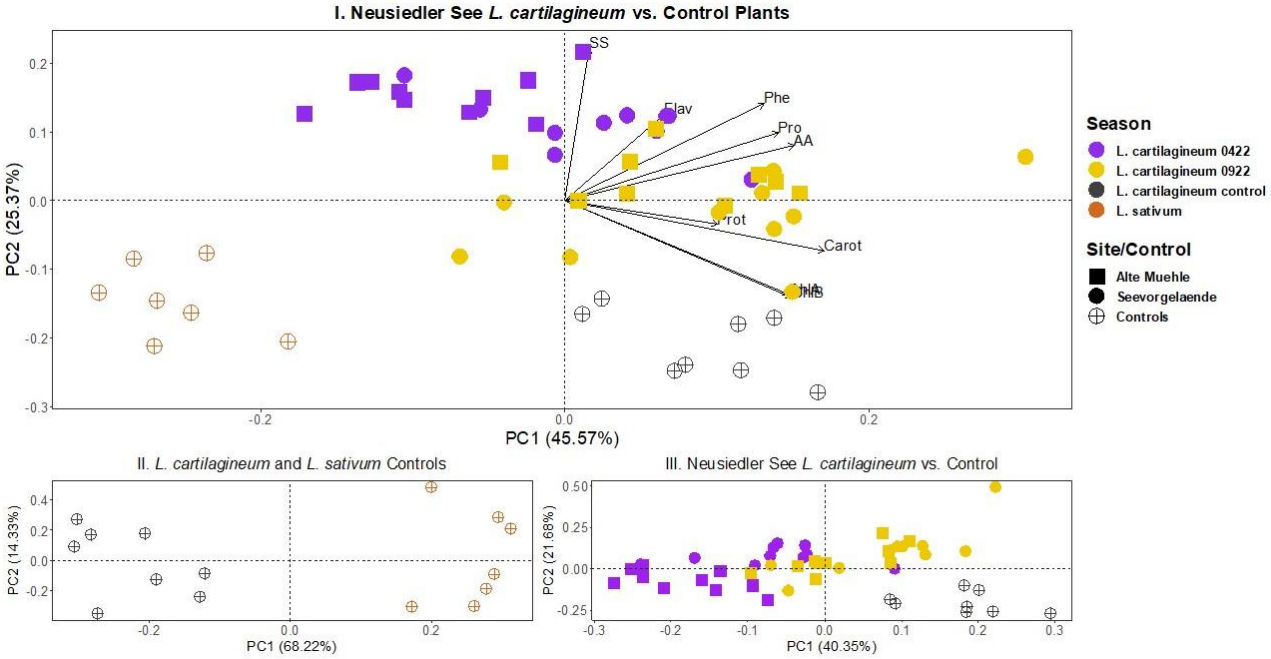


Figure 14: Principal Component Analysis (PCA) comparing *Lepidium cartilaginuem* from the Neusiedler See with control species (*L. cartilaginuem* and *L. sativum*). The large graph (**I**) captures the overall variation, showing how wild *L. cartilaginuem* across all sites (shapes) and seasons (colors) compares to the control species (color).

The smaller inset graphs: **II** provides a more detailed comparison between the control species alone; **III** focuses on the variation between *L. cartilagineum* from different sites and seasons, and the control treatments. Before conducting the PCA, data were separated into corresponding datasets.

3.4.6 Comparative analysis of metabolites in control plants: *Lepidium cartilagineum* and *Lepidium sativum*

Control plants *Lepidium cartilagineum* (LC) had a significantly higher content of all pigments compared to *Lepidium sativum* (LS) ($p < 0.05$) (Figure 15 a-c); this differentiation was also visible in PCA analysis (**Figure 14**). Further, LC also showed significantly higher phenol content (**Figure 15 d**) ($p < 0.05$). Flavonoid concentration was similar in both species, with LS having a slightly higher concentration than LC, but not significantly (**Figure 15 e**). Soluble sugars were lower in lower content in LS plants, but did not differ significantly. The highest differences were observed in amino acids and proline content (**Figure 15 g,h**), on average 12.43 times higher concentration of free amino acids and 14.63 times in proline content in LC controls than in LS ($p < 0.05$). Even though was the concentration of proteins in LC plants almost 2 times higher, it did not reveal any significant differences (**Figure 15 i**).

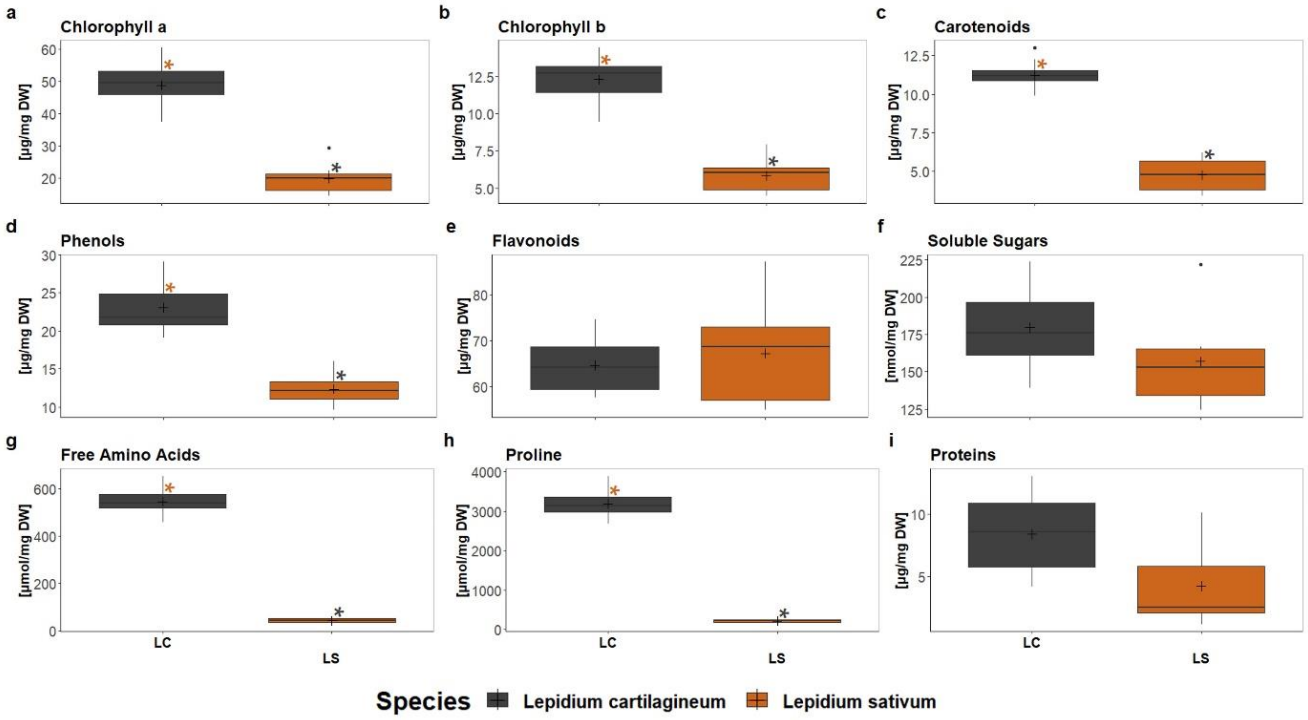


Figure 15: Boxplot representing concentrations of analyzed metabolites in control plants, *Lepidium cartilagineum* and *Lepidium sativum.* Y-axis depicting the concentration per mg of dry weight (DW). Error bars are first and fourth quartile. Medians are represented by straight-horizontal lines and mean values by "+." Vertical stars stand for significant differences found by separate post-hoc - Dunnett-tests; the color coding stands for significant relation to *Lepidium cartilagineum* (dark grey); *Lepidium sativum* (dark brown), p-value coding: $p < 0.05$ *; n = 7-8

3.4.7 Comparative analysis of metabolites of *Lepidium cartilagineum* control and wild plants

photosynthetic pigments (a, b, c)

Lepidium cartilagineum controls had overall significantly higher concentrations of chlorophyll a and b (mean = 48.89 µg/ml) than all wild *L. cartilagineum* samples ($p < 0.05$) (**Figure 16 a,b**). The concentration of carotenoids was significantly higher in the control LC compared to April replicates ($p < 0.05$) (**Figure 16 c**). There was no significant difference between the controls and the September samples. Carotenoid levels in the controls remained higher than those in AM samples from September but did not exceed the concentrations found in the samples from Seevorgelände (**Figure 16 c**).

phenols, flavonoids, soluble sugars (d, e, f)

Phenolic content, oppositely to chlorophylls, revealed significantly higher concentration in all sample groups compared to the control ($p < 0.05$) (**Figure 16 d**). Flavonoid content was also the lowest in controls (**Figure 16 e**). Significantly lower only to SV site in both seasons ($p < 0.05$). The concentration of soluble sugars (**Figure 16 f**) was significantly lower in control LC compared to wild plants ($p < 0.05$) (**Figure 16 f**).

free amino acids, proline, proteins (g ,h, i)

Free amino acids and proline did not reveal any significant differences compared to control LC (**Figure 16 g,h**). All LC species (controls and wild) were in one significance group, significantly higher than LS across both metabolites (**Supp. Table 17**). Proteins did not show any significant differences across the sample groups compared to the controls, but showed lower variance, similarly as April samples(**Figure 16 i**).

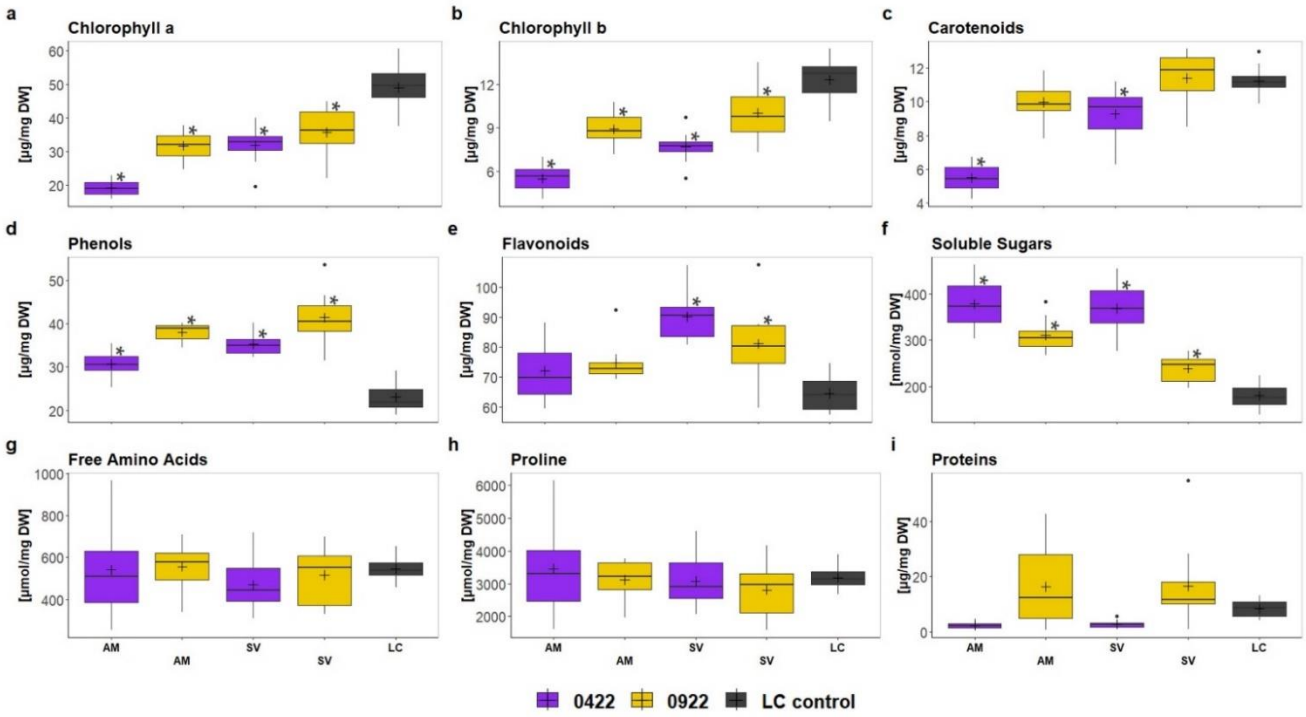


Figure 16: Boxplot representing metabolite concentrations across sites and seasons compared to controls *Lepidium cartilagineum*. X-axis depicting the site acronym (AM – Alte Mühle, SV – Seevorgelände) and the numbers are representing the 2 seasons (0422 – April , 0922 – September). Y- axis depicting the concentration per mg of dry weight (DW). Mean values are represented by "+". Vertical stars stand for significant differences found by separate post-hoc - Dunnett-tests; the colour coding stand for significance to Lepidium cartilagineum (dark-grey), p-value coding = $p < 0.05$ * ; n = 8-10.

3.5 Summary

The results revealed that both plant species thrive in alkaline soils with high sodium, carbonate, sulfate, and chloride levels. However, the L. cartilagineum root zone soil exhibited higher conductivity and ion content than *L. tenuis* (see **Tables 2** and **3**). Distinct responses to alkaline pH and high soil conductivity were observed between the species. *L. cartilagineum* showed increased photosynthetic pigments and proteins under high conductivity, while L. tenuis experienced a decrease in these metabolites but an increase in proline, free amino acids, and sugars (**Figures 9** and **10**). No significant changes in proline and amino acids were found for *L. cartilagineum* plants. Both species exhibited greater variability in metabolite content in April, with lower levels of pigments and proteins but higher levels of soluble sugars compared to September samples. *L. cartilagineum* controls generally had higher metabolite levels than non-halophytic controls, except for flavonoids (**Figure 15**).; similarly, *L. tenuis* controls, particularly those that were nitrogen-fixing, showed higher metabolite levels compared to non-halophytes model species *L. japonicus* and nitrogen-fed *L. tenuis* (**Figure 12**). Wild *L. cartilagineum* and *L. tenuis* had higher contents of soluble sugars, phenols, and flavonoids compared to all control plants. *L. cartilagineum* also displayed elevated levels of proline and free amino acids in control conditions (**Figure 16**). *L. tenuis* controls, particularly those that were nitrogen-fixing, exhibited notable differences compared to plants from April, especially those from Darscholacke and Alte Mühle. Generally, these controls had metabolite content similar to September samples (**Figure 13**).

4 Discussion

4.1 Variability of analyzed soil properties in the Neusiedler See-Seewinkel region

Soil properties, including conductivity, soil water content, and pH ($CaCl_2$), were analyzed to evaluate the influence of soil characteristics on plant growth and nutrient availability. These parameters provide insights into the soil's ability to retain water, conduct electrical charges (related to salinity), and be acidic or alkalic. Additionally, concentrations of key cations (Na^+, Ca^{2+}, Mg^{2+}, K^+) and anions (Cl^-, CO_3^{2-}, SO_4^{2-}) were measured to determine their contribution to elevated salinity levels. Soil samples were collected from the root zones of two halophyte species. The sites were categorized into "salt patches" (Alte Mühle and Seevorgelände), characterized by visible dryness and elevated salt content, predominantly inhabited by *Lepidium cartilagineum* and occasionally by *Lotus tenuis*. *Lotus tenuis* were also present at two other sites, Darscholacke and Sechsmahdlacke, categorized as "lakesides" due to their proximity to salt ponds. The sampling was conducted during two seasons, Spring – April and Autumn – September. The salinity of soil is mostly caused by salts, differing in concentration; common are NaCl, Na_2SO_4, $NaHCO_3$, Na_2CO_3, $MgSO_4$, $CaSO_4$, $CaCl_2$, $MgCl_2$, and KCl, with NaCl being usually the dominant salt. Soil salinity negatively impacts plant growth and development and is commonly connected to high Na^+ accumulation. Hence, the most common mechanism halophytes developed is low maintenance of cellular Na^+ (Chaudhary, 2019). Various mechanisms have been proposed. However, *Lepidium cartilagineum* has been shown to accumulate salt in the vacuoles to protect the cytoplasm from toxic effects and maintain cell turgor by accumulating high amounts of osmolytes such as proline (Mile et al., 2002). *Lotus tenuis* was shown to exclude toxic Na^+ and Cl^- from xylem, in saline and waterlogging environments (N. Teakle et al., 2007). Further analyzed ions such as potassium K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} are also essential nutrients for plant growth and development. Potassium is crucial for plant functions, and its balance with Na^+ is key for salinity tolerance. Calcium stabilizes cell structures and membranes in saline conditions, while Mg^{2+} supports enzyme activity and chlorophyll formation. Chloride, a micronutrient and photosynthesis co-factor, regulates pH but becomes toxic at high levels. Sulfate, essential for protein synthesis via cysteine and methionine, also inhibits plant growth at elevated concentrations and is more toxic than chloride (Chaudhary, 2019).

Our results showed that soil properties varied spatially and seasonally for both species. Specifically, soil from Darscholacke in April for *L. tenuis* plants exhibited the greatest variation in analyzed soil properties compared to other sites (**Table 2**). However, the ratio of ionic composition remained consistent across both seasons and all locations (***Table S12***). The soil water content (SWC) was similar across all sites and seasons; the soil of *L. cartilagineum* exhibited a higher SWC range than *L. tenuis*. SWC was generally consistent between Alte Mühle and Seevorgelände, regardless of plant species, and higher than at Sechsmahdlacke and Darscholacke for *L. tenuis* (**Tables 2, 3**), but no significant

differences were observed for any species. The difference in SWC can be related to differences in the soil's ability to retain water. SWC is influenced by soil particle size, with finer particles typical of clay/loam soils retaining more water than coarser soils like gravel or sand (Gardner, 1979). This might suggest that soils at sites might have differed in structure, where Seevorgelände and Alte Mühle have finer particles, resembling loam-clay, which allows them to retain more water than those at Darscholacke and Sechsmahdlacke. Besides, all sites, except Darscholacke, exhibited higher SWC in September than in April (**Tables 2, 3**). This seasonal variation might be attributed to lower precipitation rates in April (mean = 28 mm) compared to September (mean = 48 mm), and the timing of sample collection at the end of each month. The highest precipitation occurred on the 27th of September, and the 15th of April, which also corresponds to the higher values of SWC in September, as the precipitation happened near the sampling time (ZAMG, n.d.). The precipitation and temperature influence soil salinity; higher temperatures increase evapotranspiration, causing upward groundwater movement and bringing soluble salts to the surface, thus increasing salinity (Xu et al., 2013). During the sampling seasons, the average temperature was higher in September (15.4°C) than in April (9.6°C) (ZAMG, n.d.), with also higher precipitation observed in September. We observed increased SWC and conductivity in September for sites Sechsmahdlacke and Seevorgelände. Darscholacke contained higher SWC and conductivity in April, and Alte Mühle soil decreased in conductivity and ion content with higher SWC. Thus, seasonal changes in salinity did not show any specific trend regarding the SWC and differed across sites.

Soil salinity, measured by soil pore electrical conductivity (Ece), indicates the soil water's ability to conduct electrical current due to charged ions (Rhoades et al., 1989). It is classified as non-saline (0-2000 µS/cm), moderately saline (2000-4000 µS/cm), and strongly to extremely saline (above 4000 µS/cm) (Hailu & Mehari, 2021). In this study, soils surrounding the root systems of *L. cartilagineum* were strongly saline, with maximum mean values reaching 15510 µS/cm ± 4022.39 at Seevorgelände in September (**Table 3**). For *L. tenuis*, salinity was noticeably lower across all sites, with mean values not exceeding 2000 µS/cm at Alte Mühle, Seevorgelände, Sechsmahdlacke, and Darscholacke in September. The highest conductivity was measured in the soil of Darscholacke in April (5688 ± 1904.31 µS/cm), categorized as a moderately saline environment (**Table 2**). Although the soil at the remaining *L. tenuis* sites had lower conductivity, it is important to recognize that even lower salinity levels can negatively impact plant growth. This may be due to factors such as high concentrations of toxic ions, the presence of a $CaCO_3$ concretion layer that restricts root growth and water infiltration, reduced calcium availability, and decreased microbial activity, all of which can diminish soil fertility (Sharma et al., 2000). Therefore, despite the relatively low conductivity levels across the sites, these conditions may still be suitable only for plants that can tolerate such environments.

Sodium (Na^+) was the most abundant ion regardless of season, site, or plant species (**Tables 2, 3**), classifying the soil as sodic-saline (Qadir et al., 2007). Moreover, the sodium content must be significantly higher to increase conductivity because it contributes to conductivity with less strength than bivalent cations like Ca^{2+} and Mg^{2+} (Ismayilov et al., 2021). Both were found in visibly lower concentrations (**Table 3**). Sodic soils typically exhibit a higher pH, ranging from 8.5 to 11 (Stavi et al., 2021). In our analysis, the pH across all sites was alkaline, with the soil of *L. tenuis* plants exhibiting lower pH values, reaching mean values of 8.27 at Darscholacke in September (**Table 2**). *L. cartilagineum* soils had higher pH levels, with mean values reaching 8.84 at Seevorgelände in September (**Table 3**). However, all sites were either below a pH of 8.5 or at the lower end of the alkaline range. The reduction in pH could be attributed to microbial processes as well as plant processes, occurring in the root zone by releasing organic acids that can lower pH and enhance the solubility and hence availability of phosphates and other nutrients in the soil (Hoberg et al., 2005; Saghafi et al., 2019). Thus, the soil pH in the root zone can be lower than in other areas of the same region.

The anion pool is predominantly characterized by high sulfate, carbonate, and chloride in the soda pans in the Seewinkel region (Boros et al., 2014). These salt pans contain many minerals, such as sodium carbonate/soda (Na_2CO_3), Glauber's salt ($MgSO_4$), Epsom salt (Na_2SO_4), and table salt (NaCl), brought to the surface due to groundwater movement and further dissolved due to precipitation (Krachler, 2007; Zimmermann-Timm & Teubner, 2021). Our study found that the soil surrounding the rhizosphere predominantly contained carbonate, sulfate and chloride. CO_3^{2-} was the predominant anion for *L. tenuis* plants for all sites, except for Darscholacke in April, which contained ~3 times more sulfate. For *L. cartilagineum* plants, carbonate was predominant, except for Alte Mühle in September, where the sulfate content was slightly higher than carbonate but lower than chloride (**Table 3**). Generally, the carbonate content was the highest at Seevorgelände. The second most abundant ions were chloride/carbonate or chloride/sulfate, following soil characterizations (Krachler, 2007; Zimmermann-Timm & Teubner, 2021).

Our findings demonstrate considerable variability in soil properties, with spatial factors exerting a greater influence than seasonal across the same species. This suggests that the analyzed sites differ in their physiological properties and that site-specific factors such as soil texture and structure are more significant in determining water retention and salinity levels than seasonal variations. However, differences also appear within the same site, as conductivity and the concentrations of analyzed ions, such as sodium and sulfate, varied highly between plant species, even when sampled from the same location. This indicates that different plant species have evolved distinct survival strategies and adaptations to varying salinity levels. Regarding the Ehaloph database, the highest conductivity that *L. cartilagineum* was found to endure is 26900 µS/cm (*eHALOPH Lepidium cartilagineum,* n.d.) and *L.*

tenuis, since studied in laboratory conditioned, was found to tolerate maximal salinity at 450 mM NaCl (*eHALOPH Lotus Tenuis*, n.d.).

4.2 Patterns of metabolite and protein variation in *L. tenuis* and *L. cartilagineum* and their adaptations

Soil salinity severely threatens plant growth, particularly in glycophytes, less tolerant to high salinity conditions. The adaptive responses of halophytes to salt stress include complex physiological and biochemical changes, which are often reflected in the alteration of metabolite content, playing a crucial role in maintaining cellular homeostasis, protecting plant cells from oxidative damage, and ensuring continued growth under stress conditions (Zhao et al., 2021). Here, we present the changes in key metabolites, including photosynthetic pigments, phenols, flavonoids, soluble sugars, free amino acids, proline, and total proteins, in response to studied environmental conditions in halophytes *L. tenuis* and *L. cartilagineum*. Nevertheless, it is important to keep in mind that not only does salinity affect plant metabolism, but the dry environment, along with other biotic and abiotic conditions present at the sampling sites, also contribute to metabolic changes. Since we do not possess the data to evaluate the effect of these external factors, we mainly focus on soil properties as a proxy for the environmental stresses plants have to tolerate.

4.2.1 Variation in photosynthetic pigments and differential responses in *L. tenuis* and *L. cartilagineum*

Elevated salt concentrations primarily inhibit photosynthesis by exposing plants to excess energy, which leads to the over-reduction of Photosystem II (PSII) reaction centers. This results in photodamage and photoinhibition as Qiu et al. (2003) described. Salinity negatively affects photosynthetic pigment content by disrupting biosynthesis or speeding up degradation. This may be due to increased chlorophyllase activity, heightened under saline conditions, and breaking down the protein-pigment-lipid complex, leading to faster pigment loss (Bacha et al., 2017; Yan et al., 2022). For instance, in the salt-sensitive Oryza sativa (rice) genotype, chlorophyll a, b, and carotenoids decrease as salinity increases. In contrast, salt-tolerant genotypes maintain stable pigment levels, attributed to their ability to accumulate proline and anthocyanins, helping mitigate salinity's effects (Chutipaijit et al., 2011).

In our results, the halophyte *L. tenuis* showed lower pigment levels at the April Darscholacke and Alte Mühle sites, with Darscholacke having the lowest pigment content. Notably, the soil at Darscholacke exhibited significantly higher conductivity and toxic ion levels than the other sites. This indicates a correlation between increased salinity and ion toxicity and the reduction in photosynthetic pigments (**Table 2**). By September, pigment content at Darscholacke had increased, corresponding with a

decrease in conductivity and toxic ion levels (**Table 2**). Redundancy analysis further supported this, showing a negative correlation between conductivity, ions (Mg^{2+} and Na^{+}), and pigment content (**Figure 9**). These findings suggest that elevated soil salinity reduces photosynthetic pigments in salt-sensitive species and some halophytes. This aligns with the study by Barbafieri et al., (2023), which showed that prolonged and high exposure to salinity (NaCl) leads to the degradation of photosynthetic pigments, even in species typically adapted to saline environments. As for *L. tenuis* from Darscholacke, the decreasing content of photosynthetic pigments can be attributed to elevated salinity and a higher toxic ion content than other sites (**Table 2**). That is not the case for *L. tenuis* from Alte Mühle in April, revealing similarly low pigment content as plants from Darscholacke but substantially lower conductivity and ion content (**Table 2**). For *Lotus tenuis,* it was shown that SWC of 15% can induce drought stress (García & Mendoza, 2014). In that regard, plants from other sites, such as Sechsmahdlacke and Seevorgelände, would also experience lower photosynthetic pigment content, influenced by lower water availability. However, neither the Alte Mühle nor Darscholacke sites exhibited the lowest soil water content (SWC); the lowest mean SWC was observed at Sechsmahdlacke (7.7%), where plants had proportionally higher pigment content (**Figure 2**). This suggests that it is not solely soil water content and salinity but rather the availability of water to plants that plays a crucial role. Even when SWC is higher in some soils, plant water uptake may be restricted by soil structure. Poor soil structure, including compaction or drainage, can limit root access to available water (O'Geen, 2013). Hence, the soil structure at Alte Mühle might be affecting the plant's ability to uptake water, resulting in drought stress and low pigment content.

Interestingly, *L. cartilagineum* also revealed the lowest pigments at Alte Mühle in April (**Figure 6**). However, the redundancy analysis for *L. cartilagineum* plants showed positively correlated pigments and conductivity, which was the case for Seevorgelände (**Table 3**); with higher seasonal conductivity also, the pigments increased. The differences in soil for *L. cartilagineum* between sites were minimal, and the reduction of pigment content might be due to lower salinity and potassium content. As mentioned by Albert et al. (2020) *L. cartilagineum* stunted growth when cultivated in garden soil and exhibited very low levels of K^{+} and other inorganic ions. This adaptation was attributed to their evolutionary development in environments with consistently high salt concentrations, where a 'free influx' of ions for osmotic adjustment is ensured. As a result, when grown in non-saline substrates, these plants cannot achieve adequate osmotic adjustment through the active, energy-intensive uptake of other cations, such as potassium (K^{+}). The potassium content in the soil at Alte Mühle was lower than at Seevorgelände, as was the conductivity (**Table 2**). This lower potassium level, potentially below the optimal range needed, may contribute to the plant's reduced ability to uptake K+, which requires more energy. However, our results support this theory only when comparing samples from different sites within the same season. The data from Alte Mühle across seasons do not fully align with this theory, as both conductivity and potassium levels decreased in September, while plants exhibited higher pigment

levels compared to April. Thus, rather than low K^+ content, it is likely that the optimal salinity level or limited water uptake, as seen in *L. tenuis*, may cause drought stress and result in reduced pigment content.

We observed lower pigment levels in April for both species across all sites and higher photosynthetic pigment content in September, with reduced spatial differentiation. Hence, seasonal effects can influence pigment content, such as other abiotic factors or stages of plant development. One of the most influencing abiotic factors on photosynthetic pigment content is high light intensity, causing the accumulation of reactive oxygen species, resulting in photooxidation of pigments (Pizarro & Stange, 2009). Based on meteorological station data from the region of Neusiedler am See, the light intensity was longer in April (192h) compared to September (165h), (ZAMG, n.d.) However, it is also established that carotenoids are important in absorbing light, scavenging ROS during photosynthesis, protecting light-harvesting proteins, and stabilizing thylakoid membranes, and tend to increase in higher content when plants are experiencing light stress (Hasanuzzaman et al., 2020). This was also confirmed in a study on halophytes, where salt stress decreased chlorophyll levels in most species. In contrast, light stress caused an increase in some carotenoids, such as violaxanthin, β-carotene, and zeaxanthin (Fitzner et al., 2023). Our findings do not suggest such a phenomenon, as all pigments were lower in April, including carotenoids (**Figure 2**). On the other hand, we did not analyze specific carotenoids and therefore cannot conclude that, in particular, those involved in ROS scavenging are not elevated. However, the lower pigment content observed earlier in the year in both species could also be linked to their developmental stage. Older plants tend to be more salt-resistant than younger ones, as they have already established themselves in the environment and adjusted through increased osmotic potential (Al Hassan et al., 2017). The analyzed plants, both perennial species, exhibit distinct flowering periods: *L. tenuis* blooms from June to August (*Lotus Tenuis - Burgenland Flora*, n.d.), while *Lepidium cartilagineum* flowers from May to October (*Lepidium Cartilagineum - Burgenland Flora*, n.d.). Thus, the salt-tolerance adjustments these plants develop over time may also contribute to the increased pigment levels observed later in life (September).

Adaptations of wild halophytes compared to controls in the content of photosynthetic pigments

A comparison of environmental samples with the controls revealed that the highest metabolite differences are between halophytes and non-halophytes (**Figure 11, 14**). Furthermore, our results suggest halophytes without saline treatment perform better than non-halophytic controls (**Figures 12, 15**). Specifically, *L. tenuis*, a nitrogen-fixing plant, exhibited the highest pigment content compared to nitrogen-fed plants and the *L. japonicus* treatments (**Figure 12**). Additionally, the photosynthetic pigment content of *L. cartilagineum* was higher than that of *L. sativum* (**Figure 15**). Grigore et al. (2012)

demonstrated that although halophytes are typically found in salt-rich environments, they exhibit increased length and greater fresh and dry weight when grown in non-saline soil. This suggests that halophytes have a low requirement for salt for growth and may prefer environments where their adaptive capabilities reduce competition for nutrients. Thus, water and nutrient limitations, rather than the presence of salt, are the key factors influencing their performance in natural settings.

Halophytes often develop thicker, succulent leaves in saline environments due to larger vacuoles, smaller intercellular spaces, and reduced chlorophyll content. However, when grown in non-saline soil, they produce thinner leaves with increased chloroplast numbers and higher chlorophyll content. Succulence in saline environments has been observed not only in *Lepidium cartilagineum* but also in *Lotus corniculatus* (Grigore et al., 2014b). Our findings align with those of Grigore et al. (2014b); *L. cartilagineum* controls exhibited higher chlorophyll content and similar carotenoid levels compared to wild samples, particularly in Seevorgelände and Alte Mühle during September (**Figure 16**). In *L. tenuis*, chlorophyll and carotenoid levels were generally higher in September. Additionally, in April, samples from Sechsmahdlacke and Seevorgelände resemble the N-fix controls. In contrast, the April samples from Darscholacke and Alte Mühle had similar or lower photosynthetic pigment content than the N-fed controls (**Figure 13**). These results suggest that halophytes perform best at their optimal salinity level, declining performance as salinity exceeds their tolerance (Rabhi et al., 2012). Interestingly, in April, Darscholacke and Alte Mühle plants showed lower pigment content than N-fed plants. As a legume, *L. tenuis* likely benefits from symbiosis with rhizobacteria, as seen in control plants with higher pigment levels than N-fed ones. Early symbiosis with arbuscular mycorrhiza and rhizobacteria has improved *L. tenuis* salinity and water stress tolerance. In a study with soil salinity of 5.1 dS/m (similar to Darscholacke in April) and an exchangeable sodium percentage of 56%, the plants reduced Na^+ content and tolerated salinity and water stress. Hence, early symbiosis is key for nutrient competition and survival in low-fertility soils (García & Mendoza, 2014). This suggests that plants at Darscholacke and Alte Mühle in April likely lacked beneficial symbiosis, which may have been established by September, as indicated by reduced sample variability and improved pigment levels across all September samples.

Overall, both halophytes in a controlled environment showed higher chlorophyll a, b, and carotenoid content than wild samples. *L. tenuis* N-fix samples exhibited the highest pigment content, similar to samples from September. They differed more from samples from April, particularly of Alte Mühle and Darscholacke, revealing similarities to N-fed plants. Hence, we can conclude that Alte Mühle and Darscholacke sites were the least favorable for analyzed halophytes, which might have been due to lower nutrient uptake due to soil properties, lower microbial activity, but also other environmental factors, such as pathogens, herbivores, and soil temperature, which are not included in this study. Also, at Darscholacke, higher salinity might have been above levels of tolerance of *L. tenuis*, causing low pigment content. Additionally, because both plants revealed a lower content of pigments in April, this

suggests that younger species, even under seemingly favorable conditions, are still developing tolerance and forming a salt-tolerant community. Nevertheless, seasonal differences can also be attributed to varying soil properties to which plants are adapted. For example, *L. cartilagineum* performs better in highly saline environments, while *L. tenuis* may struggle due to the incomplete establishment of necessary symbiosis mechanisms in younger plants.

4.2.2 Seasonal and spatial stability of phenols and flavonoids in wild halophytes with elevated levels compared to controls

Phenolics are in plants involved in signaling, hormone control, and reproduction, but they also are formed as a response of plants to various environmental stresses such as drought, high light, cold, and salinity; they are mostly involved in ROS scavenging (Ray et al., 2024). In halophytes, increased salinity reduces both dry and fresh plant weights but raises the levels of phenols and flavonoids. However, at optimal salinity, these compounds may decrease. The ideal salinity and plant response vary by species. Pungin et al. (2023) showed that halophytes react differently to increased salinity. For example, *G. maritima* had higher phenolic content as salinity increased, while *S. marina* initially showed a decrease, followed by an increase at the highest salinity level (500 mM $CaCl_2$). Our results showed that *L. tenuis* plants had consistent phenol content across different locations and seasons (**Figure 1, 3 I, II-d**). This was also visible in redundancy analysis, revealing a very low influence on the content of phenols by any of the analyzed soil properties. The antioxidative activity of halophytes is mainly attributed to their adaptation mechanism to produce phenols and flavonoids in saline environments (Bueno et al., 2020). However, our results showed that the total phenol content did not elevate, even in the environment with the highest salinity (Darscholacke April) and lowest water content (Sechsmahdlacke September). Phenol levels in *L. cartilagineum* followed a similar pattern to pigment levels and were positively linked to conductivity (**Figure 5, 10**). At the Seevorgelände site, higher salinity led to increased phenol content, while at Alte Mühle, phenol levels decreased as salinity rose between April and September (**Figure 7 I, II-d).** Rather than conducitvity, pH had a positive and strong effect on phenols, with the highest pH recorded at Seevorgelände, especially in September. This suggests that *L. cartilagineum* thrives better at Seevorgelände, increasing phenol production due higher pH and conductivity. The rise in phenols, which boosts antioxidative activity, could also explain the elevated pigment content in September.

Flavonoids are similar to all phenolic compounds, are formed when plants are experiencing abiotic and biotic stresses, and are mainly involved in neutralizing reactive oxygen species (Ray et al., 2024). Principal component analysis (PCA) revealed that flavonoids significantly contributed to spatial variability in both plant species (**Figures 1, 5**). In *L. cartilagineum*, we observed only a spatial significant pattern and lower flavonoid content in April in samples from Alte Mühle than from Seevorgelände (**Figure 7 I-e**). *Lepidium cartilagineum* plants were positively influenced by

conductivity and negatively by analyzed ions (**Figure 10**). *L. cartilagineum* showed higher flavonoids and phenols at Seevogelände, suggesting its adaptive mechanism to oxidative stress, possibly caused by higher salinity compared to Alte Mühle. Flavonoid content showed greater variability than phenols in *L. tenuis* plants. This variation was largely attributed to elevated flavonoid levels at Darscholacke across both seasons (**Figure 3 I-e**). A seasonal increase in flavonoid content was observed in September for plants from Alte Mühle and Seevogelände (**Figure 3 I, II-e**). Conductivity and elevated ion content positively influenced flavonoid content in *Lotus tenuis* plants (**Figure 9**), visible in plants from Darscholacke in April. Furthermore, in legumes are flavonoids often expressed as signaling molecules to initiate symbiosis with bacteria or mycorrhizal fungi in low nutrient content (Abdel-Lateif et al., 2012). Our findings show that *L. tenuis* plants from the Darscholacke region, which had the lowest pigment levels and highest conductivity, also exhibited the highest flavonoid content. This increased flavonoid concentration may respond to oxidative stress from elevated salinity and other abiotic stressors. Alternatively, it could result from the plants' lack of symbiotic activity, with flavonoids potentially attracting symbiotic partners to improve their salt-stress tolerance. However, this pattern does not apply universally to all April samples or those from Alte Mühle and Seevorgelände. Hence, the increased flavonoid content could be due to either the absence of symbiosis or a stress response from established symbiosis. Our results did not show any clear spatial or seasonal trends in response to salinity, ionic content, soil water content (SWC), or pH across species. Only increase of phenolics due to higher pH in *L. cartilagineum*, but this wasn´t observed in flavonoids content. However, we observed species-specific differences. Similar to Pungin et al. (2023), our results indicate that halophyte responses to stress are species-specific rather than driven by seasonal or spatial factors. Further analysis, such as gas chromatography, is needed to determine whether elevated salinity, pH or ionic content caused the increase in phenols or flavonoids. It is also important to acknowledge that beyond drought and salinity, other factors such as herbivory, cold, and elevated temperatures might contribute to phenols and flavonoid disturbances (Bibi et al., 2022).

Adaptations of halophytes in total phenol and flavonoid content compared to controls

L. tenuis nitrogen-fixing controls showed a higher phenol content than *L. japonicus* and *L. tenuis* nitrogen-fed controls (**Figure 12d**). However, phenol levels were significantly lower compared to wild *L. tenuis* controls in both nitrogen-fixing and nitrogen-fed treatments (**Figure 13d**). A similar pattern was observed in *L. cartilagineum*, where the control group exhibited significantly higher phenol content than the non-halophyte *Lepidium sativum*, yet still lower than that of wild *L. cartilagineum*. These results are in line with Qasim et al. (2017), who suggest elevated phenol content may respond to environmental stresses such as salinity compared to controls with optimal nutrient conditions. Therefore, differences in phenol levels between sites and across seasons may suggest that salinity does not elevate phenol content or that levels are lower at certain sites, particularly for *L. cartilagineum*.

However, compared to control conditions, phenol content is indeed elevated in both species, suggesting their response to environmental factors by increasing phenol production.

In Principal component analysis, both flavonoids and phenols were positively correlated to each other in wild samples and negatively to control plants (**Figure 11, 14**). Controls of *L. tenuis* showed higher flavonoids, concretely N-fix, most substantially differing from N-fed *L. japonicus* (**Figure 12**). Interestingly, *L. sativum* revealed a similar content of flavonoids to *L. cartilagineum* **(Figure 15**). Regarding wild samples, both controls had a lower content of flavonoids. The lowest differences in flavonoid content to controls were found at the Alte Mühle site for both species. *L. sativum* is an edible plant used in medicine due to its many health-promoting benefits, including a high flavonoid content (Painuli et al., 2022). Hence, the flavonoid content of *L. sativum* cannot be considered an increase, as it is already high even in optimal, non-stressful conditions. However, control plants also remained lower in flavonoid content when compared to wild samples of both species.

Despite minimal seasonal and spatial variation in phenol and flavonoid content, both species exhibited elevated levels of these compounds compared to controls under optimal conditions. This suggests that increased phenol and flavonoid content in plants from Neusiedler See-Seewinkel may significantly affect their salt tolerance. Further analyses are needed to identify which specific phenolics contribute to this salt-tolerance mechanism.

4.2.3 Seasonal variability of soluble sugars and comparable response in controls for halophytes

Soluble sugars in plants participate in various metabolic processes and serve as signaling molecules that regulate different genes, mainly those related to photosynthesis, sucrose metabolism, and osmolyte synthesis (Rosa et al., 2009). Accumulation of osmolytes, such as proline and soluble carbohydrates, in response to elevated salinity has also been reported in the halophyte *Thellungiella halophila*. Increased salt content led to the accumulation of starch and total sugars in the leaves, as well as up-regulation of genes that encode enzymes involved in sugar metabolism, suggesting an important role of soluble sugars in plant salt tolerance (Wang et al., 2013). Our findings showed that halophytes *L. tenuis* and *L. cartilagineum* experienced higher variability and overall higher content of soluble sugars in April compared to September (**Figure 3, 7 II-f**). However, for *L. tenuis*, a significant increase in soluble sugar content was observed only at the sites of Sechmahdlacke and Seevorgelände. Concerning elevated salinity and the accumulation of soluble sugars, this effect was confirmed specifically for *L. tenuis* from Sechsmahdlacke, where the soluble sugar content was notably higher in April and the correlated content of Na^+ was higher. However, this trend was not observed for plants from Seevorgelände, where increased salinity decreased soluble sugar content (**Table 2, Figure 9**). SWC and pH also negatively

influenced soluble sugars, where SWC wasn´t significant, but pH was the lowest observed in April at Sechsmahdlacke. However, pH in soil for *L. tenuis* from Seevorgelände was not significantly lower in April (**Table 2**). The accumulation of soluble sugars in plants from Sechsmahdlacke in April may be attributed to higher salinity, increased Na^+ content, lower pH, and reduced soil water content compared to September. Still, this is inconsistent with the results from Seevorgelände, where the opposite trend was observed. Furthermore, Alte Mühle and Darscholacke's biggest seasonal changes in soil properties did not lead to variations in soluble sugars. Symbiosis with plant-growth-promoting bacteria, such as rhizobacteria, has been demonstrated to enhance photosynthesis and stress tolerance in plants by increasing the production of soluble carbohydrates (Su et al., 2024). Recent studies have shown that plants inoculated with rhizobacteria exhibit improved adaptation to water deficits, largely due to elevated levels of soluble sugars and other osmolytes under such conditions (Amine-Khodja et al., 2022). This suggests that plants from Seevorgelände and Sechsmahdlacke might benefit from effective rhizobial symbiosis and have higher sugar content, consistent with their higher chlorophyll levels. In contrast, plants at Darscholacke and Alte Mühle, where rhizobial symbiosis seems less effective, also showed lower sugar content.

L. cartilagineum plants also revealed a seasonally higher content of sugars in April compared to September. Higher conductivity was observed in samples from Alte Mühle in April. However, chloride content might have played a more important role, as its increase significantly increased levels of soluble sugars, particularly at Alte Mühle in April (**Figure 10**). Still, this was not confirmed for *L. cartilagineum* from Seevorgelände, which, on the other hand, exhibits the highest levels of sodium correlated to conductivity (***Table S7***). Murakeözy et al. (2003) revealed that *L. cartilagineum* had the highest content of reducing sugars and sucrose in April and decreased later in the season, suggesting that plants early in the season cope with the salinity and environmental stresses by producing more osmolytes, such as soluble sugars. This might also be attributed to plants of *L. tenuis*, which, although not significantly overall, revealed a higher sugar content in April (**Figure 4 I,II-f**).

Adaptations of halophytes compared to controls in total soluble sugar content

Soluble sugars in control plants showed no significant differences across both species, with *L. tenuis* N-fix controls displaying slightly elevated sugar content compared to other controls (**Figure 15, 12 f**). Both species from Seewinkel exhibited significantly higher soluble sugar content than the controls not subjected to saline stress (**Figure 12, 15 f**). *L. tenuis* plants from April and Alte Mühle, Seevorgelände in September had a higher content of soluble sugars than both controls; Darscholacke and Sechsmahdlacke only differed from N-fed plants. Therefore, it may appear that environmental conditions, such as salinity, did not significantly increase soluble sugars during certain seasons or at

specific sites compared to controls. The differences and elevation in carbohydrate levels were still noticeable compared to controls.

These findings suggest that soluble sugars are crucial for osmoprotection, helping plants manage salinity and other abiotic stresses, and are reduced in non-saline soils. The higher soluble sugar content in April may be linked to the plant's developmental stage and enhanced osmoprotection. Additionally, as a legume, *L. tenuis* showed greater spatial variation in April, with plants from Sechsmahdlacke and Seevorgelände having higher sugar content and improved photosynthetic pigments, likely due to more effective symbiosis compared to the Alte Mühle and Darscholacke sites. In contrast, *L. cartilagineum* prefers soils with higher carbonate, typical of Seevorgelände, and showed lower soluble sugar content as soil carbonate increased.

4.2.4 Patterns of variation in free amino acids and proline content in both halophytes and their adaptations

Amino acids are the building blocks for protein synthesis and play a crucial role in plant development and metabolism. Beyond their role in protein synthesis, their accumulation has been shown to benefit plants under various abiotic stresses (Ashraf & Foolad, 2007). In halophytes like *S. persica*, amino acids such as proline, methionine, cysteine, and phenylalanine accumulate under high NaCl conditions, while others, like glutamic acid, lysine, leucine, and isoleucine, decrease—mainly due to their roles in energy metabolism, as precursors for stress-signaling molecules, and their participation in the biosynthesis of secondary metabolites essential for osmoprotection, ROS scavenging, and stress signaling (Kumari & Parida, 2018). Therefore, various amino acids play distinct roles in stress responses, and lower content does not necessarily indicate reduced stress response.

In our results on *L. tenuis* plants, we found free amino acids and proline to be correlated to each other and to be in higher content in plants from Sechsmahdlacke and Seevorgelände in April (**Figure 1, Figure 4 I,II-g,h).** The content of free amino acids and proline was, however, rather weakly influenced by conductivity and ions and negatively by pH and soil water content (**Figure 9**), which have been the lowest at Sechsmahdlacke and Seevorgelände (only Sechsmahdlacke significantly). Besides elevated amino acids, plants from Seevorgelände and Sechsmahdlacke in April also revealed a higher content of sugars and photosynthetic pigments, suggesting their mechanism and osmoprotection to cope with environmental stresses. It is also important to note that *L. tenuis,* as a legume, is actively getting ammonium from the symbiont when in symbiosis with rhizobacteria, which is then used for amino acid formation. Hence, symbiotically active plants are shown to be rich in amino acids (Amine-Khodja et al., 2022). Accumulation of some amino acids in nodules, such as proline, glutamine, arginine, Gamma-aminobutyric acid (GABA), and histidine, was shown to be associated with osmoregulation in

Medicago sativa plants when inoculated with rhizobia and exposed salt-stress (Bertrand et al., 2016). However, the accumulation of amino acids in leaves was more connected to salt response rather than induced by symbiosis. Therefore, it is disputable whether the amino acids found in *L. tenuis* leaves are due to more efficient symbiosis at Sechsmahdlacke and Seevorgelände or simply a salt-coping mechanism used for osmoregulation early in the season. This might also explain why, in September, there were no significant differences in amino acid and sugar content, as plants may have better adjusted and coped with stress.

L. cartilaginuem plants, on the other hand, did not reveal any seasonal or spatial variation in free amino acids or proline content. Popp & Albert (1980) analyzing the content of amino acids in halophytes from Neusiedler See found that *L. cartilaginuem* plants exhibited the highest content of proline than all other plants and that the total content of amino acids was by 70% of proline. Further, it was found that 20% of all nitrogen in *L. cartilagineum* is utilized for proline synthesis; hence, the osmotic regulation in *Lepidium cartilagineum* is mostly attributed to very high proline content. A high accumulation of proline was also later confirmed by Murakeözy et al. (2003). Higher proline content in L. cartilagineum species with steady content from March to August was found.

Adaptations of halophytes compared to controls in total free amino acids and proline content

N-fixing control plants of *L. tenuis* had the highest proline and free amino acid content, while *L. japonicus* showed slightly higher levels in N-fed plants, though not significantly. Symbiosis can enhance free amino acid and proline content, as ammonia, typically produced through nitrogen fixation, is more efficiently utilized by plants for amino acid synthesis compared to nitrate (Atilio J. & Causin, 1996). Additionally, plants from Sechsmahdlacke and Seevorgelände in April displayed similar amino acid levels to nitrogen-fixing plants, suggesting that the increase was due to established symbiosis. Conversely, plants from Alte Mühle and Darscholacke exhibited a trend similar to N-fed plants, as seen with other metabolites, indicating lower amino acid content likely due to reduced symbiosis. *Lepidium cartilagineum*, also in control conditions, revealed a much higher proline and free amino acid content than *L. sativum* (**Figure 15**). Further comparison to wild samples revealed that *L. cartilagineum* exhibits a higher proline and amino acid content, resulting from an inherent ability to adjust to high salt content rather than induced osmotic stress.

4.2.5 Seasonal pattern in the total content of proteins in wild halophytes

Identifying proteins in halophytic plants is important for understanding how they tolerate higher salinity levels. The proteins involved in salt response are typically associated with photosynthesis, ROS scavenging, osmotic adjustments, membrane signaling, carbohydrate synthesis, and other processes

involved in plants growth and development (Zhang et al., 2012). Salinity generally decreases protein levels in salt-stressed plant parts due to reduced protein synthesis and increased protein hydrolysis. In some cases, protein levels may rise due to the synthesis of new salt-induced proteins or reduced proteolysis (Dubey, 1999). Our results found seasonal changes more influential on protein content in both halophytes (**Figures 4** and **8 I,II-i**). Plants in early seasons had substantially lower total protein content than in September. Further, we also detected the lowest protein content in plants from Darscholacke in April, which was also lower than plants from other sites. Plants degrade proteins along with lipids, nucleic acids for resource allocation, the mobilized molecules are then further transferred to the other parts of plant where they are needed (Araújo et al., 2011). Which was also proposed in leaves of Yacon vegetable plants, exhibited a decrease in protein content and increased content of carbohydrates and phenolics as they developed through maturity (Simon et al., 2024). However, in our results, the more mature plants had a higher content of proteins, whereas plants in the early season exhibited a higher content of amino acids (except plants from Darscholacke). Thus, the lower content of proteins can be rather explained by the higher susceptibility of younger plants coping with salinity and environmental conditions. Hence being more susceptible to protein degradation and photosynthetic apparatus damage (Dubey, 1999). Their later establishment and adapted salt tolerance might be the reason why their protein content and the content of photosynthetic pigments increased. The response of salt-tolerance in halophytes can be observed in the increase of content of proteins related to photosynthesis, such as chlorophyll a/b binding proteins (CAB), RuBisCO activase (RCA), involved in the regulation of light reaction, CO_2 assimilation resulting in higher photosynthetic activity (Zhang et al., 2012). We do not possess the results of the exact proteins that were up-regulated and down-regulated in our plants. However, we observed a correlation between protein content and the content of analyzed pigments in PCA (**Figure 1,5**). This might be due to enhanced photosynthetic activity and higher salt tolerance in the latter season. Plants from Darscholacke exhibited a lower content of amino acids in September, along with a lower content of proteins, suggesting that they were still adjusting to elevated salinity in April. In Lepidium plants, the content was influenced mainly by conductivity and pH (**Figure 10**). Conductivity did not reveal significant effects in *L. cartilagineum* plants. However, pH was the lowest in April for both sites, also having effects on pigments phenols and flavonoids and negative on sugars, indicating that lower pH might not be suitable for *L. cartilagineum* plants and prefer more alkaline soils. Negative impacts on proteins had ions, sodium in *L. tenuis* and chloride in *L. cartilaginuem*, mostly in plants from April, suggesting their higher accumulation hence possible protein degradation in the early season (Dubey, 1999).

Adaptations of halophytes compared to controls in total protein content

Control plants did not differ in protein content across treatments and species (**Figure 12, 14**). *L. tenuis* during September exhibited higher protein content than both controls, while plants from April did not

differ from controls. Besides, plants from Darscholacke in April had significantly lower proteins than *L. tenuis* N-fix (**Figure 13**). When plants are not exposed to salinity or environmental stresses, their protein content does not change across differing species. However, the higher protein content in plants from September suggests an improved response to environmental conditions and enhanced tolerance. In *L. cartilagineum*, although no significant differences to controls were found, plants from September had, on average, higher content of proteins (**Figure 15, 16**). As visible on photosynthetic pigments, higher protein content in September in both species might be due to their better-established tolerance to given environmental conditions.

4.3 Limits and future perspectives

As mentioned, both plants have a differing mechanism in tolerance to salinity*, L. tenuis* plants have been shown to tolerate increased salinity by the exclusion of both sodium and chloride via xylem, and *Lepidium cartilagineum* as audiophile by accumulation and storage of high ammount of sodium in vacuoles, and as also visible in our results, stabilization of membrane and osmotic regulation due to high accumulation of proline (Mile et al., 2002; N. Teakle et al., 2007). Also, *L. cartilagineum* was as well shown to prefer soils rich in sulfate ions (Albert & Kinzel, 1973). However, our results do not suggest this trend. As plants seemed to perform better in the soils lower in sulfate, and richer in carbonate such as those in the Seevorgelände area. It would, therefore be necessary to identify which ions accumulate the most in the plants, determine the relative ionic composition in their leaves, and investigate ionic transport and homeostasis using methods proposed by Al Hassan et al. (2017) and Weimberg, (1987).

Under oxidative stress, in addition to elevated levels of phenols, flavonoids, and non-protein amino acids, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) are also typically upregulated as a defense mechanism (Hasanuzzaman et al., 2020). As the relative content of these compounds has been shown to fluctuate in plants exposed to various stress factors—such as changes in water availability, light intensity, temperature, and increased salinity across different species—this could provide valuable insights into which stressors induce the most oxidative stress in the analyzed plants. Given that the total amount of flavonoids and phenols showed only minimal variation in response to fluctuating salinity and water conditions between sites and seasons, it would be important to investigate other mechanisms that plants use to adapt to changing environmental conditions.

Further insights into proteins involved in salt tolerance and their up- or down-regulation under specific environmental conditions would help us understand the mechanisms employed by plants. By examining proteins that are up-regulated in response to salt stress, we can better understand their osmoregulatory

mechanisms, such as those involved in sugar metabolism or proline synthesis (Δ^1-pyrroline-5-carboxylate, P5C). Amino acids and proline also exhibit similar responses, but various amino acids can play different roles in plants. Therefore, it is important to determine which amino acids are present in higher amounts. Some, such as ornithine and arginine, play crucial roles in proline synthesis. Identifying whether other amino acids, besides proline, are also important in osmoregulation could provide a better understanding of this mechanism (Hayat et al., 2012).

Additionally, as mentioned by Forlani & Funck (2020) the ninhydrin method has been shown to erroneously detect related molecules such as ornithine, hydroxyproline, and D-proline, leading to overestimating proline content. Therefore, we suggest employing GC-MS methods to more accurately identify and quantify the amino acids present in the samples.

All plants exhibited seasonal variability in metabolites; however, the seasonal variability in the analyzed soil properties was less pronounced, with noticeable changes mainly observed at Darscholacke for *L. tenuis*. We speculated about the symbiotic status of *L. tenuis* plants and its potential role in enhancing stress tolerance. However, for *L. cartilagineum*, a symbiotic partner that could improve its metabolic status has not yet been identified. To determine whether changes in metabolite composition are related to plant-growth-promoting bacteria, environmental stress factors, or differences in plant developmental stages and possible metabolic shift, the next steps would be to analyze plant-microbiome composition from the roots and the surrounding rhizosphere using metagenomic sequencing and to conduct time-course studies examining plants at different developmental stages and their shifts in metabolite profiles.

It is also important to note that some outliers, particularly in the first three replicates, were not excluded from the analysis. This could have influenced the metabolite content, especially proteins in *L. cartilagineum* plants (***Table S29***). Additionally, analyzing plants from different seasons independently may have introduced seasonal variability that does not align with the results of soil properties. Therefore, we recommend conducting a more randomized analysis of plants from both seasons to better account for these factors.

5 Conclusion

This study examined the seasonal and spatial variability in soil properties and plant metabolites. We found that spatial variability was generally higher in April than September for metabolites and soil properties. Specifically, Darscholacke exhibited the highest ion content and salinity, while the soil water content (SWC) and pH did not reveal big differences. Overall, the soils were sodic-saline, with sodium as the dominant cation and chloride, sulfate, and carbonate as the predominant anions. Soil conductivity varied between the two plants, with *L. cartilagineum* tolerating more saline conditions. However, the ionic ratio of the soil remained consistent across all sites and seasons for both species. Where Alte Mühle site revealed similar contents of all anions. Plants did not exhibited a clear response pattern to the analyzed soil properties. *L. cartilagineum* showed a higher preference for the Seevorglände site, with elevated salinity and ionic content. In contrast, *L. tenuis* appeared more susceptible to elevated salinity, displaying reduced levels of photosynthetic pigments and proteins at Darscholacke site, although this was also observed at sites with substantially lower salinity. Thus, other abiotic or biotic factors, not included in this study, likely contributed to metabolite differences in both halophytes. In regard the metabolic changes, *L. tenuis* plants from Darscholacke had the lowest levels of photosynthetic pigments, proteins, soluble sugars, free amino acids, and proline but the highest flavonoid content. These plants also showed higher similarities to N-fed controls. By September, were all plants, including *L. tenuis* from Darscholakce more closely aligning to N-fixing controls, suggesting improved performance and symbiotic efficiency later in the season. *L. cartilagineum* plants also showed seasonal variability, with higher pigment and protein content in April and higher soluble sugars and flavonoids. Alte Mühle plants had the lowest pigment and phenol content but the highest soluble sugars. Despite lower salinity on the site of Alte Mühle, plants revealed a lower content of pigments, suggesting that differences in soil structure, herbivory, pathogens, and competition have influenced plant performance at various sites. Another factor that could cause seasonal metabolic changes in both halophytes is their coping mechanism, which improves towards their maturity (September). Furthermore, both halophytic control species in non-saline soils showed better performance and higher content of photosynthetic pigments, phenols, flavonoids (only *L. tenuis*), free amino acids, and proline compared to non-halophyte model species. Suggesting their developed strategies to accumulate more osmoprotectants and metabolites involved in oxidative protection, even in a not-stressed environment. *L. tenuis* N-fix performed the best, showing the advantage of a symbiotic partner. Furthermore, both wild halophytes exhibited higher levels of phenols, flavonoids, and soluble sugars than controls, indicating adaptations to oxidative stress and osmotic adjustments in environmental conditions. *L. cartilagineum* maintained high levels of free amino acids and proline, even in non-saline environment, indicating an inherent osmotic regulation mechanism.

The study highlights that both plant species exhibit significant seasonal and site-specific variations in metabolite content and stress responses. The findings also suggest the importance of rhizobacterial symbiosis in improving salt-stress tolerance, particularly for *L. tenuis*. However, the relationship between soil properties and plant metabolism was not always clear, indicating a need for further research into plant metabolic changes related to life cycle and environmental conditions.

Appendix

Zusammenfassung (Deutsch)

Die Region Neusiedler See–Seewinkel in Österreich ist ein semiarides Gebiet mit einem pannonischen Klima, das durch heiße, trockene Sommer und kalte Winter gekennzeichnet ist. Die Überreste des Pannonischen Meeres führten zur Bildung eines salzführenden Horizonts und damit zu stark salinen und alkalischen Böden. Nur Pflanzen mit spezialisierten Anpassungen können unter diesen herausfordernden Bedingungen gedeihen. In unserer Studie haben wir verschiedene Metaboliten, wie Chlorophyll a und b, Carotinoide, Phenole, Flavonoide, lösliche Zucker, freie Aminosäuren, Prolin und Gesamtproteine, untersucht, um die metabolischen und proteinhaltigen Anpassungen dieser Pflanzen in der salinen und trockenen Umgebung zu identifizieren. Wir untersuchten zwei Halophyten, *Lepidium cartilagineum* (Brassicaceae) und *Lotus tenuis* (Fabaceae), über zwei Jahreszeiten (April und September) an den Standorten Seewinkel: Alte Mühle und Seevorgelände. Zusätzlich wurde *L. tenuis* auch an Darscholacke und Sechsmahdlacke gesammelt. Diese Studie gibt einen Überblick über die Umweltbedingungen an verschiedenen Standorten und in zwei Jahreszeiten. Sie betrachtet dabei die Bodenmerkmale wie Bodenfeuchtigkeit, pH-Wert, ionische Zusammensetzung (Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, CO_3^{2-}, SO_4^{2-}) und Leitfähigkeit rund um das Wurzelsystem der untersuchten Pflanzen zum Zeitpunkt der Probenahme.

Wir fanden saisonale und räumliche Variabilität in der Leitfähigkeit und dem Ionengehalt; das ionische Verhältnis war jedoch an allen Standorten und für beide Arten ähnlich. Alle Böden waren von Natrium dominiert und auf der Anionenseite von Carbonat/Sulfat/Chlorid. Beide Pflanzen waren unterschiedlichen Ionenkonzentrationen und Salinitätsniveaus ausgesetzt, wobei der Boden im Wurzelbereich von *L. cartilagineum* eine höhere Salinität und mehr Ionen (Na^+, Cl^-, CO_3^{2-}, SO_4^{2-}) aufwies, während *L. tenuis* niedrigere Salinitätswerte bevorzugte. *L. cartilagineum* erwies sich als am besten geeignet für die salineren Böden, was sich in erhöhten Fotosynthesepigmenten und Proteinen widerspiegelte. *L. cartilagineum* akkumulierte auch hohe Mengen an Aminosäuren, insbesondere Prolin, selbst in nicht-salinem Gartenboden (Kontrolle), was auf einen inhärenten osmotischen Regulationsmechanismus hinweist. Beide Arten zeigten im April niedrigere Pigment- und Proteingehalte als im September, was wahrscheinlich auf eine weniger effektive Salztoleranz in der frühen Lebensphase der Pflanzen zurückzuführen ist. Für *L. tenuis* könnte dies auch auf eine weniger effektive Symbiose zu Beginn der Saison hinweisen. Besonders deutlich wurde dies bei den Pflanzen aus Darscholacke, die im April schwach abschnitten, sich aber bis September verbesserten. Sie zeigten höhere Pigment- und Proteingehalte, näherten sich den stickstofffixierenden Kontrollen an und wiesen weniger Schwankungen bei Zucker- und Aminosäuren auf. Diese symbiotische Anpassung hilft *L. tenuis* wahrscheinlich, sich später in der Saison besser an salzige Bedingungen anzupassen und zeigt

einen möglichen Anpassungsmechanismus für salzige Umgebungen. Kontrollpflanzen hatten mehr Chlorophyll und Carotinoide. Das deutet darauf hin, dass Wildpflanzen aus dem Neusiedler See-Seewinkel ihre Pigmente bei Salztstress aufgrund der großen Vakuolen reduzieren. In nicht-salinen Umgebungen erhöhen sie die Pigmente, um die Photosynthese zu verbessern. Beide Pflanzen hatten im Vergleich zu den Kontrollen höhere Gehalte an löslichen Zuckern, Phenolen und Flavonoiden. Das deutet darauf hin, dass ihre osmotischen und antioxidativen Anpassungen zu einem Mechanismus der Salztoleranz führten.

Table of Contents

Supplementary Material

Supplementary Tables of PCA and RDA

Lotus RDA & PCA

Table S1 Permanova results of RDA

Lotus tenuis: The permutation ANOVA for the entire RDA model, including axes, terms, and margins of soil properties, showed that all criteria were significant.

		Df	Variance	F	Pr(>F)	Signif.codes
Model	Model	6	4.689714	3.082747	0.001	***
	Residual	17	4.310286			
		Df	**Variance**	**F**	**Pr(>F)**	
Axes	RDA1	1	1.745754	6.885348	0.003	**
	RDA2	1	1.163872	4.590372	0.023	*
	RDA3	1	1.087946	4.290916	0.021	*
	RDA4	1	0.530325	2.09163	0.376	
	RDA5	1	0.128841	0.508156	0.963	
	RDA6	1	0.032976	0.130061	0.992	
	Residual	17	4.310286	0	0	
		Df	**Variance**	**F**	**Pr(>F)**	
Terms	pH_CaCl2	1	0.823636	3.248464	0.002	**
	SWC_BD	1	0.494814	1.951574	0.072	
	conductivi	1	1.326657	5.232408	0.001	***
	Na	1	0.807102	3.183254	0.009	**
	Mg	1	0.721981	2.84753	0.013	*
	CO3	1	0.515524	2.033252	0.062	
	Residuals	17	4.310286			
		Df	**Variance**	**F**	**Pr(>F)**	
Margin	pH_CaCl2	1	1.000298	3.945228	0.002	**
	SWC_BD	1	0.405417	1.598988	0.165	
	conductivi	1	0.65045	2.565409	0.022	*
	Na	1	0.538994	2.125821	0.058	
	Mg	1	0.834255	3.290347	0.007	**
	CO3	1	0.515524	2.033252	0.062	
	Residuals	17	4.310286			
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Table S2 Summary table of RDA

Lotus tenuis, RDA summary (left) containing the eigenvalues, explained proportion of variance and cumulative proportion. Frame value represents R-squared. Sum of PCs´ cumulative proportion represents unexplained variance by our model (residuals). In the right-side table are loadings (eigenvectors), used soil properties, and depicted their correlation to individual axes.

	Eigenvalues	Proportion Explained	Cumulative Proportion
RDA1	1.74575416	0.193972684	0.193972684
RDA2	1.163871627	0.12931907	0.323291754
RDA3	1.087945577	0.120882842	0.444174596
RDA4	0.530324906	0.05892499	0.503099586
RDA5	0.128840931	0.014315659	0.517415245
RDA6	0.032976421	0.003664047	0.521079291
PC1	1.613897244	0.179321916	0.700401207
PC2	1.099374604	0.122152734	0.822553941
PC3	0.691379798	0.076819978	0.899373919
PC4	0.505193475	0.056132608	0.955506527
PC5	0.231700503	0.0257445	0.981251027
PC6	0.092299218	0.010255469	0.991506496
PC7	0.041452622	0.004605847	0.996112343
PC8	0.026990121	0.002998902	0.999111245
PC9	0.007998792	0.000888755	1
Constrained variance ; R2 = 0.5211			
R^2 adjusted = 0.3520			
Unconstrained variance ; sum of PCs = 0.4789			

	pH_CaCl2	SWC_BD	conductivity	Na	Mg	CO3
RDA1	0.538436284	0.382727	-0.589665096	-0.365868327	-0.633771876	-0.000353659
RDA2	0.237385974	0.039174	0.437088371	-0.014263001	0.254986524	-0.493169529
RDA3	0.451965327	0.51437	0.575635023	0.615852958	0.695793899	0.453628235
RDA4	-0.170422375	-0.29768	-0.256843506	-0.631346297	-0.006669152	-0.461975994
RDA5	0.066753651	0.305119	-0.216196975	-0.259330936	0.094721532	-0.506657129
RDA6	-0.644950986	0.636942	-0.131085593	-0.144261529	-0.200422767	0.284397344

Table S3: Correlation between analyzed soil properties

	Correlation between soil properties *L. tenuis*									
	pH_CaCl2	**SWC_BD**	**conductivity**	**Mg**	**Ca**	**Na**	**K**	**Cl**	**SO4**	**CO3**
pH_CaCl2	1	0.108155	0.160312362	0.170482	0.114191	0.261287	-0.17325	0.264265	0.296791	-0.05075
SWC_BD	0.108155	1	0.014529686	0.028552	0.040549	0.193119	0.238202	0.064144	0.060452	0.377955
conductivity	0.160312	0.01453	1	0.893195	0.834831	0.801146	0.373178	0.946458	0.924699	0.236687
Mg	0.170482	0.028552	0.893195092	1	0.979143	0.665307	0.405726	0.813233	0.929712	0.088194
Ca	0.114191	0.040549	0.834830911	0.979143	1	0.549483	0.358582	0.724539	0.846904	0.031304
Na	0.261287	0.193119	0.801146489	0.665307	0.549483	1	0.543064	0.921503	0.864017	0.668563
K	-0.17325	0.238202	0.373177667	0.405726	0.358582	0.543064	1	0.423889	0.434831	0.596928
Cl	0.264265	0.064144	0.946457565	0.813233	0.724539	0.921503	0.423889	1	0.937359	0.39227
SO4	0.296791	0.060452	0.924699484	0.929712	0.846904	0.864017	0.434831	0.937359	1	0.261743
CO3	-0.05075	0.377955	0.236687052	0.088194	0.031304	0.668563	0.596928	0.39227	0.261743	1

Table S4 PCA loadings and summary

First one (**Figure 5**) is representing the eigenvectors and summary to the principal component analysis of metabolites originating from Neusiedler See. All others belong to **Figure 16**, **I** display the summary results and eigenvector correlations for the model of metabolites from Neusiedler See's *Lotus tenuis* and the controls (*Lotus tenuis* and *Lotus japonicus*) across both treatments; N-fix and N-fed. **II** stands for comparison of only control species across both treatments. **III** belongs to analysis of only *Lotus tenuis* from Neusiedler See and controls from growth chambers across both treatments.

Figure 1	***L. tenuis* Neusiedler See**	**Components**								
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	0.4981	-0.1396	0.0819	0.3316	0.1191	-0.1221	-0.1596	0.1104	-0.7410
	Chlorophyll b	0.4456	-0.1026	0.1714	0.2825	0.5404	-0.0919	0.1394	-0.3587	0.4827
	Carotenoids	0.4251	-0.2984	0.0006	0.1190	-0.4826	0.0540	-0.3042	0.4405	0.4400
	Phenols	0.2375	-0.0107	-0.5705	-0.5464	0.4117	0.0007	-0.3767	0.0883	0.0145
	Flavonoids	0.0603	-0.1717	-0.7584	0.3057	-0.2418	-0.1088	0.4389	-0.1871	-0.0175
	Soluble Sugars	0.1561	0.5415	-0.1525	0.2796	-0.0971	0.6775	-0.2490	-0.2247	0.0042
	Amino Acids	0.3485	0.4474	0.0483	-0.2019	0.0640	0.0572	0.6143	0.5005	0.0081
	Proline	0.3096	0.4164	0.0743	-0.2536	-0.4194	-0.5314	-0.1220	-0.4332	0.0063
	Proteins	0.2662	-0.4296	0.1794	-0.4699	-0.2084	0.4660	0.2715	-0.3655	-0.1538
	Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Standard deviation	1.8198	1.4056	1.1423	0.9081	0.7694	0.6777	0.5305	0.4713	0.1674
	Proportion of Variance	0.3680	0.2195	0.1450	0.0916	0.0658	0.0510	0.0313	0.0247	0.0031
	Cumulative Proportion	0.3680	0.5875	0.7325	0.8241	0.8899	0.9409	0.9722	0.9969	1.0000
Figure 11	**I Neusiedler See *L. tenuis* vs. Control Plants**	**Components**								
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	0.4224	-0.2784	0.1065	-0.3785	0.0362	0.0676	0.0167	0.1087	-0.7559
	Chlorophyll b	0.3830	-0.2672	0.1631	-0.3969	-0.3899	-0.3524	0.2414	-0.2365	0.4553
	Carotenoids	0.3915	-0.3375	-0.0505	-0.0294	0.4267	0.4562	-0.2059	0.3053	0.4513
	Phenols	0.3529	0.2448	-0.3995	0.2066	-0.1240	-0.3911	0.1749	0.6437	0.0028
	Flavonoids	0.2767	0.1900	-0.5787	-0.1045	0.3917	-0.2148	-0.1799	-0.5556	-0.0289
	Soluble Sugars	0.2887	0.5136	-0.1140	-0.0754	-0.3828	0.6477	0.2196	-0.1401	0.0181
	Free Amino Acids	0.3250	0.3168	0.4201	0.1512	-0.1690	-0.1589	-0.7352	-0.0159	0.0076
	Proline	0.2649	0.2756	0.5140	0.2579	0.5017	-0.1131	0.4942	-0.1202	-0.0027
	Proteins	0.2478	-0.4483	-0.1066	0.7414	-0.2626	0.0802	0.0559	-0.2859	-0.1279
	Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Standard deviation	2.0496	1.2630	1.1968	0.8096	0.6521	0.5604	0.4475	0.3933	0.1489
	Proportion of Variance	0.4668	0.1773	0.1591	0.0728	0.0473	0.0349	0.0223	0.0172	0.0025
	Cumulative Proportion	0.4668	0.6440	0.8031	0.8760	0.9232	0.9581	0.9804	0.9975	1.0000
Figure 11	**II *L. tenuis* and *L. japonicu* s Controls**	**Components**								
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	-0.3704	0.3917	-0.0481	-0.2166	-0.1957	0.0688	-0.0479	0.1177	0.7753
	Chlorophyll b	-0.4032	0.2669	0.0236	-0.0115	-0.2110	0.1722	0.7550	-0.0793	-0.3391
	Carotenoids	-0.3525	0.4342	-0.0447	-0.2356	-0.0992	-0.0535	-0.5953	-0.1462	-0.4912
	Phenols	-0.3427	-0.2339	0.4596	0.2701	0.1664	0.6807	-0.2103	-0.0826	0.0395
	Flavonoids	-0.3338	-0.0552	0.6117	0.1741	0.1101	-0.6724	0.0334	0.1224	0.0259
	Soluble Sugars	-0.3552	-0.3175	-0.2782	-0.3061	0.3092	0.0424	0.0123	0.6957	-0.1427
	Free Amino Acids	-0.3526	-0.3267	-0.2980	-0.1968	0.3798	-0.1703	0.0712	-0.6660	0.1396
	Proline	-0.1920	-0.5558	-0.0764	-0.0176	-0.7918	-0.0755	-0.1187	-0.0346	-0.0158
	Proteins	-0.2443	0.1128	-0.4875	0.8133	0.0285	-0.1038	-0.0925	0.0890	0.0204
	Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Standard deviation	2.2554	1.2642	0.9702	0.8248	0.7051	0.3290	0.2386	0.1618	0.0698
	Proportion of Variance	0.5652	0.1776	0.1046	0.0756	0.0552	0.0120	0.0063	0.0029	0.0005
	Cumulative Proportion	0.5652	0.7428	0.8474	0.9230	0.9782	0.9902	0.9966	0.9995	1.0000
Figure 11	**III Neusiedler See *L. tenuis* vs. Control**	**Components**								
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	0.4570	-0.2684	0.0491	0.3699	-0.0089	-0.0339	-0.0746	-0.1201	0.7474
	Chlorophyll b	0.4127	-0.2576	0.1095	0.3681	0.4169	0.3118	-0.1381	0.3098	-0.4792
	Carotenoids	0.4049	-0.3183	-0.1300	0.0061	-0.4423	-0.4175	0.0754	-0.3903	-0.4357
	Phenols	0.3065	0.3506	-0.3973	-0.2315	0.2025	0.3946	-0.3036	-0.5315	-0.0162
	Flavonoids	0.1998	0.2843	-0.5815	0.2017	-0.4107	0.1830	0.2802	0.4682	0.0250
	Soluble Sugars	0.2383	0.5452	-0.0058	0.1041	0.3149	-0.6876	-0.1834	0.1709	-0.0193
	Free Amino Acids	0.3465	0.2827	0.4145	-0.1580	0.1107	0.1348	0.7443	-0.1377	0.0002
	Proline	0.2832	0.2118	0.4965	-0.2718	-0.5041	0.1733	-0.4570	0.2479	0.0009
	Proteins	0.2569	-0.3667	-0.2331	-0.7236	0.2351	-0.1309	0.0502	0.3550	0.1436
	Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Standard deviation	1.8916	1.3574	1.2407	0.8428	0.7096	0.6137	0.4737	0.4463	0.1606
	Proportion of Variance	0.3976	0.2047	0.1711	0.0789	0.0560	0.0419	0.0249	0.0221	0.0029
	Cumulative Proportion	0.3976	0.6023	0.7734	0.8523	0.9082	0.9501	0.9750	0.9971	1.0000

Lepidium RDA & PCA

Table S5 Permanova results of RDA
Permutation ANOVA of the whole RDA model, axes, terms, and margins of soil properties. All criteria were significant.

		Df	Variance	F	Pr(>F)	Signif.code
Model	Model	6	6.766731	2.524972	0.024	*
	Residual	5	2.233269			
		Df	**Variance**	**F**	**Pr(>F)**	
Axes	RDA1	1	4.240648	9.494262	0.018	*
	RDA2	1	1.39385	3.12065	0.433	
	RDA3	1	0.724488	1.622034	0.765	
	RDA4	1	0.337915	0.756547	0.945	
	RDA5	1	0.064467	0.144333	0.996	
	RDA6	1	0.005363	0.012007	1	
	Residual	5	2.233269			
		Df	**Variance**	**F**	**Pr(>F)**	
Terms	pH_CaCl2	1	1.575795	3.528001	0.036	*
	SWC_BD	1	0.472186	1.057163	0.377	
	conductivi	1	0.766527	1.716154	0.181	
	Mg	1	1.846757	4.13465	0.009	**
	Cl	1	1.412796	3.163066	0.041	*
	SO4	1	0.69267	1.550798	0.211	
	Residuals	5	2.233269			
		Df	**Variance**	**F**	**Pr(>F)**	
Margin	pH_CaCl2	1	1.012843	2.267623	0.105	
	SWC_BD	1	0.435392	0.974786	0.45	
	conductivi	1	0.683535	1.530346	0.225	
	Na	1	1.152052	2.579295	0.072	
	Mg	1	0.243915	0.546093	0.728	.
	CO3	1	0.69267	1.550798	0.211	
	Residuals	5	2.233269			
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Table S6 Summary table of RDA

Lepidium cartilagineum, RDA summary (left) containing the eigenvalues, explained proportion of variance and cumulative proportion. Frames value represents R-squared. Sum of PCs´ cumulative proportion represents unexplained variance by our model (residuals) which is explained only by 5 PCs. In the right-side table are loadings (eigenvectors), used soil properties, and depicted their correlation to individual axes.

	Eigenvalues	Proportion Explained	Cumulative Proportion
RDA1	4.240648498	0.471183166	0.471183166
RDA2	1.393850395	0.154872266	0.626055433
RDA3	0.724487764	0.08049864	0.706554073
RDA4	0.337914516	0.037546057	0.74410013
RDA5	0.064466782	0.007162976	0.751263106
RDA6	0.005362883	0.000595876	0.751858982
PC1	1.562461574	0.173606842	0.925465823
PC2	0.378254881	0.04202832	0.967494143
PC3	0.218746618	0.02430518	0.991799323
PC4	0.049362605	0.005484734	0.997284057
PC5	0.024443485	0.002715943	1
Constrained variance ; R2 = 0.7519			
R^2 adjusted = 0.4534			
Unconstrained variance ; sum of PCs = 0.2481			

	pH_CaCl2	SWC_BD	conductivi	Mg	Cl	SO4
RDA1	0.575882	-0.07585	0.392741	0.050701	-0.47889	-0.33294
RDA2	0.059729	-0.07815	-0.04852	0.529605	0.226189	0.418956
RDA3	-0.43211	-0.51706	0.162952	0.133601	-0.05363	-0.10062
RDA4	0.129776	0.240149	-0.61691	0.616103	0.289894	-0.05245
RDA5	-0.59627	0.618243	-0.06616	-0.31985	-0.5587	-0.56956
RDA6	-0.32511	0.529988	-0.65718	0.466061	-0.56608	-0.61348

Table S7 Correlation between analyzed soil properties

Correlation between soil properties *L. cartilagineum*										
	pH_CaCl2	**SWC_BD**	**conductivity**	**Mg**	**Ca**	**Na**	**K**	**Cl**	**SO4**	**CO3**
pH_CaCl2	1	-0.3347	0.325904604	0.122249	0.109225	0.316416	-0.03677	0.315688	0.409021	0.115914
SWC_BD	-0.3347	1	-0.647608347	0.082904	0.174896	-0.60752	-0.15859	-0.52943	-0.64532	-0.28725
conductivity	0.325905	-0.64761	1	-0.64922	-0.73175	0.988148	0.73128	0.022347	0.305722	0.864305
Mg	0.122249	0.082904	-0.649221679	1	0.985935	-0.66518	-0.67201	0.181821	0.055499	-0.70645
Ca	0.109225	0.174896	-0.731750316	0.985935	1	-0.7344	-0.73912	0.195557	0.01941	-0.76419
Na	0.316416	-0.60752	0.9881484	-0.66518	-0.7344	1	0.70158	0.044207	0.312885	0.869716
K	-0.03677	-0.15859	0.731279625	-0.67201	-0.73912	0.70158	1	-0.624	-0.39186	0.934389
Cl	0.315688	-0.52943	0.022346903	0.181821	0.195557	0.044207	-0.624	1	0.909883	-0.44092
SO4	0.409021	-0.64532	0.305722167	0.055499	0.01941	0.312885	-0.39186	0.909883	1	-0.19433
CO3	0.115914	-0.28725	0.864304556	-0.70645	-0.76419	0.869716	0.934389	-0.44092	-0.19433	1

Table S8 PCA loadings and summary

Lepidium cartilagineum, Principal component analysis results. First one (**Figure 12**) is representing the eigenvectors and summary to the principal component analysis of metabolites originating from Neusiedler See. All others belong to **Figure 16**, **I** display the summary results and eigenvector correlations for the model of metabolites from Neusiedler See's *Lepidium cartilagineum* and the controls (*Lepidium cartilagineum* and *Lepidium sativum*). **II** stands for comparison of only control species. **III** belongs to analysis of only *Lepidium cartilagineum* from Neusiedler See and controls from growth chambers.

Figure 5	***L. cartilagineum*** **Neusiedler See**					Components				
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	0.43176	-0.08916	0.08325	-0.32884	0.32061	0.03927	0.38969	0.61774	-0.22972
	Chlorophyll b	0.44062	-0.08231	-0.04506	0.08129	0.32878	0.20580	-0.79854	0.03889	-0.02858
	Carotenoids	0.44356	-0.11101	-0.01515	-0.25004	0.26835	0.04538	0.30946	-0.67637	0.31760
	Phenols	0.39756	-0.03065	0.00727	0.09661	-0.70009	0.55369	0.08603	0.09309	0.13728
	Flavonoids	0.20659	-0.26926	0.72849	-0.06690	-0.29534	-0.47213	-0.14952	-0.07843	-0.10489
	Soluble Sugars	-0.30489	-0.09103	0.61134	0.22366	0.34097	0.58516	0.11790	-0.04807	0.01029
	Free Amino Acids	0.15194	0.66448	0.11763	-0.06280	-0.04195	0.09168	0.01746	-0.29557	-0.64790
	Proline	0.07897	0.66981	0.26492	-0.04688	0.05820	-0.12624	-0.06489	0.23323	0.62833
	Proteins	0.31571	0.03233	-0.04800	0.86762	0.13544	-0.24041	0.25796	0.02275	-0.03183
	Summary	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
	Standard deviation	2.107375	1.40266048	1.037147738	0.834886457	0.663051833	0.487775671	0.306752926	0.185463172	0.112897
	Proportion of Variance	0.49345	0.21861	0.11952	0.07745	0.04885	0.02644	0.01046	0.00382	0.00142
	Cumulative Proportion	0.49345	0.71205	0.83157	0.90902	0.95787	0.98431	0.99476	0.99858	1
Figure 14	**I Neusiedler See** ***L. cartilagineum*** **vs. Control Plants**					Components				
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	0.383463	-0.353619	-0.040659778	0.333628279	-0.151985047	-0.126273782	-0.142268876	0.742281775	-0.068422
	Chlorophyll b	0.388603	-0.366414	0.039851496	0.102614438	-0.300206012	-0.061008742	0.693817744	-0.353749469	0.047363
	Carotenoids	0.444091	-0.190509	0.116289322	0.227164276	-0.009105489	0.294129105	-0.616738384	-0.4803959	0.060174
	Phenols	0.341618	0.370173	0.200768009	-0.055904139	0.218606837	0.701457286	0.284672621	0.264698852	0.109088
	Flavonoids	0.17178	0.331684	0.602294221	0.350377325	0.335608811	-0.500373862	0.064840676	-0.06469417	-0.059649
	Soluble Sugars	0.041747	0.581086	-0.00761621	0.172677102	-0.786696958	-0.018138066	-0.083698157	-0.009099256	-0.066423
	Free Amino Acids	0.392856	0.211318	-0.433505073	-0.159071256	0.208704277	-0.117276436	0.021221171	-0.094221542	-0.7219
	Proline	0.365863	0.259433	-0.453727228	-0.127831355	0.142499375	-0.326634495	-0.038152282	-0.004803655	0.669444
	Proteins	0.26188	-0.087959	0.431643673	-0.793225943	-0.208605156	-0.175019323	-0.15445433	0.099320041	-0.017029
	Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Standard deviation	2.025165	1.511184066	1.093237755	0.849540206	0.567871859	0.524365596	0.25311038	0.169814995	0.088335
	Proportion of Variance	0.4557	0.25374	0.1328	0.08019	0.03583	0.03055	0.00712	0.0032	0.00087
	Cumulative Proportion	0.4557	0.70944	0.84224	0.92243	0.95826	0.98881	0.99593	0.99913	1
Figure 14	**II** ***L. cartilagineum*** **and L. sativum controls**					Components				
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	-0.39504	0.05565354	-0.055852853	-0.064678964	-0.413213068	-0.2998677	0.144304639	0.735428313	-0.105824
	Chlorophyll b	-0.393052	0.071233917	-0.034007327	0.020211572	-0.507682773	-0.114412645	0.420427524	-0.621377824	0.071897
	Carotenoids	-0.397207	0.051573909	-0.105110658	-0.095603718	-0.0930398	-0.208411453	-0.852341952	-0.197720916	0.041312
	Phenols	-0.358371	-0.243275405	0.201198305	-0.22407719	0.627801455	-0.517102996	0.229109928	-0.084763636	-0.022131
	Flavonoids	0.05943	-0.739734664	0.509340903	-0.217114954	-0.327827003	0.149251124	-0.112911755	0.007976025	0.011601
	Soluble Sugars	-0.396061	0.019046079	-0.043934045	-0.17467607	0.147619948	0.552711494	0.033015708	-0.030854064	-0.693547
	Free Amino Acids	-0.396216	0.03428203	-0.027030907	-0.183821864	0.164293239	0.502468696	0.068941465	0.151773754	0.70715
	Proline	-0.19363	0.337484363	0.741307706	0.535607311	0.050812251	0.056907414	-0.069571118	0.037701029	-0.010836
	Proteins	-0.219943	-0.517053714	-0.364100004	0.73330837	0.099427083	0.051760297	0.00255717	0.035834785	0.010717
	Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Standard deviation	2.477917	1.135514115	0.949910224	0.663836353	0.390880281	0.240886888	0.116615507	0.053648661	0.015359
	Proportion of Variance	0.68223	0.14327	0.10026	0.04896	0.01698	0.00645	0.00151	0.00032	0.00003
	Cumulative Proportion	0.68223	0.8255	0.92575	0.97472	0.9917	0.99814	0.99965	0.99997	1
Figure 14	**III Neusiedler See** ***L. cartilagineum*** **vs. Control**					Components				
	Loadings	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Chlorophyll a	0.481883	-0.085657597	-0.142235331	0.317618975	0.128045399	0.115443614	-0.052201705	0.761261973	-0.165442
	Chlorophyll b	0.491558	-0.021387091	-0.14527485	0.046486115	0.229473585	0.250298247	0.688840375	-0.370748877	0.08593
	Carotenoids	0.471061	0.174124879	-0.155597881	0.215881397	-0.131134656	0.198551793	-0.650192387	-0.416784511	0.154557
	Phenols	0.001687	0.632819841	0.076037756	-0.127257387	-0.589932234	0.349435892	0.218749211	0.221137393	0.103352
	Flavonoids	-0.051538	0.605982795	-0.086853814	0.481161498	0.193972972	-0.566897063	0.117550908	-0.078643246	-0.109854
	Soluble Sugars	-0.410027	0.23079505	0.089307097	0.232791295	0.538319842	0.642209886	-0.103954681	-0.002674475	-0.058383
	Free Amino Acids	0.215684	-0.000890651	0.660381322	0.044199705	-0.090011458	0.040645181	-0.009648524	-0.173239737	-0.689611
	Proline	0.141074	-0.016772049	0.690368378	0.14805322	0.136975858	-0.106756468	0.006062486	0.1134613	0.661981
	Proteins	0.258955	0.375048457	0.020989452	-0.724276511	0.459838771	-0.125062025	-0.16558358	0.106475429	-0.037638
	Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
	Standard deviation	1.905678	1.396830899	1.368113775	0.847129765	0.682803934	0.481715035	0.277060118	0.189023037	0.130872
	Proportion of Variance	0.40351	0.21679	0.20797	0.07974	0.0518	0.02578	0.00853	0.00397	0.0019
	Cumulative Proportion	0.40351	0.62031	0.82828	0.90801	0.95981	0.9856	0.99413	0.9981	1

ANOVA, p-values and summary statistics results

Lotus summary tables, one-way ANOVA, Tukey HSD & Dunnett's Test

Table S9 Soil properties Tukey HSD p-values

Spatial comparison April			Spatial comparison September			Seasonal comparison		
SWC			SWC			SWC		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.993	LT.AM922	LT.DL922	0.524	LT.AM422	LT.AM922	0.302
LT.AM422	LT.SML422	0.607	LT.AM922	LT.SML922	0.978	LT.DL422	LT.DL922	0.477
LT.AM422	LT.SV422	1	LT.AM922	LT.SV922	1	LT.SML422	LT.SML922	0.1
LT.DL422	LT.SML422	0.463	LT.DL922	LT.SML922	0.741	LT.SV422	LT.SV922	0.358
LT.DL422	LT.SV422	0.989	LT.DL922	LT.SV922	0.523			
LT.SML422	LT.SV422	0.632	LT.SML922	LT.SV922	0.978			
pH H2O			pH H2O			pH H2O		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.356	LT.AM922	LT.DL922	0.666	LT.AM422	LT.AM922	0.133
LT.AM422	LT.SML422	0.826	LT.AM922	LT.SML922	0.973	LT.DL422	LT.DL922	0.798
LT.AM422	LT.SV422	0.999	LT.AM922	LT.SV922	0.988	LT.SML422	LT.SML922	0.799
LT.DL422	LT.SML422	0.804	LT.DL922	LT.SML922	0.439	LT.SV422	LT.SV922	0.0781
LT.DL422	LT.SV422	0.3	LT.DL922	LT.SV922	0.833			
LT.SML422	LT.SV422	0.756	LT.SML922	LT.SV922	0.879			
Conductivity			Conductivity			Conductivity		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.0137	LT.AM922	LT.DL922	0.738	LT.AM422	LT.AM922	0.36
LT.AM422	LT.SML422	0.996	LT.AM922	LT.SML922	0.0663	LT.DL422	LT.DL922	0.0198
LT.AM422	LT.SV422	0.835	LT.AM922	LT.SV922	0.146	LT.SML422	LT.SML922	0.102
LT.DL422	LT.SML422	0.0103	LT.DL922	LT.SML922	0.273	LT.SV422	LT.SV922	0.0116
LT.DL422	LT.SV422	0.00471	LT.DL922	LT.SV922	0.526			
LT.SML422	LT.SV422	0.922	LT.SML922	LT.SV922	0.941			
pH CaCl2			pH CaCl2			pH CaCl2		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.609	LT.AM922	LT.DL922	0.0832	LT.AM422	LT.AM922	0.832
LT.AM422	LT.SML422	0.422	LT.AM922	LT.SML922	0.242	LT.DL422	LT.DL922	0.208
LT.AM422	LT.SV422	0.998	LT.AM922	LT.SV922	0.96	LT.SML422	LT.SML922	0.0133
LT.DL422	LT.SML422	0.0789	LT.DL922	LT.SML922	0.864	LT.SV422	LT.SV922	0.951
LT.DL422	LT.SV422	0.512	LT.DL922	LT.SV922	0.0418			
LT.SML422	LT.SV422	0.512	LT.SML922	LT.SV922	0.125			
Magnesium			Magnesium			Magnesium		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.00236	LT.AM922	LT.DL922	0.168	LT.AM422	LT.AM922	0.471
LT.AM422	LT.SML422	1	LT.AM922	LT.SML922	0.862	LT.DL422	LT.DL922	0.0415
LT.AM422	LT.SV422	0.997	LT.AM922	LT.SV922	0.981	LT.SML422	LT.SML922	0.517
LT.DL422	LT.SML422	0.00231	LT.DL922	LT.SML922	0.0564	LT.SV422	LT.SV922	0.625
LT.DL422	LT.SV422	0.00294	LT.DL922	LT.SV922	0.101			
LT.SML422	LT.SV422	0.997	LT.SML922	LT.SV922	0.975			
Sodium			Sodium			Sodium		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.127	LT.AM922	LT.DL922	0.997	LT.AM422	LT.AM922	0.251
LT.AM422	LT.SML422	0.99	LT.AM922	LT.SML922	0.0236	LT.DL422	LT.DL922	0.00251
LT.AM422	LT.SV422	0.996	LT.AM922	LT.SV922	0.0795	LT.SML422	LT.SML922	0.0789
LT.DL422	LT.SML422	0.0833	LT.DL922	LT.SML922	0.0311	LT.SV422	LT.SV922	0.835
LT.DL422	LT.SV422	0.173	LT.DL922	LT.SV922	0.105			
LT.SML422	LT.SV422	0.951	LT.SML922	LT.SV922	0.821			
Chloride			Chloride			Chloride		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.0368	LT.AM922	LT.DL922	0.895	LT.AM422	LT.AM922	0.401
LT.AM422	LT.SML422	0.989	LT.AM922	LT.SML922	0.0822	LT.DL422	LT.DL922	0.0102
LT.AM422	LT.SV422	0.984	LT.AM922	LT.SV922	0.209	LT.SML422	LT.SML922	0.236
LT.DL422	LT.SML422	0.0242	LT.DL922	LT.SML922	0.217	LT.SV422	LT.SV922	0.634
LT.DL422	LT.SV422	0.0226	LT.DL922	LT.SV922	0.491			
LT.SML422	LT.SV422	1	LT.SML922	LT.SV922	0.905			
Calcium			Calcium			Calcium		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.0206	LT.AM922	LT.DL922	0.489	LT.AM422	LT.AM922	0.227
LT.AM422	LT.SML422	0.999	LT.AM922	LT.SML922	0.415	LT.DL422	LT.DL922	0.144
LT.AM422	LT.SV422	0.999	LT.AM922	LT.SV922	0.706	LT.SML422	LT.SML922	0.0529
LT.DL422	LT.SML422	0.0245	LT.DL922	LT.SML922	0.0568	LT.SV422	LT.SV922	0.997
LT.DL422	LT.SV422	0.0239	LT.DL922	LT.SV922	0.122			
LT.SML422	LT.SV422	1	LT.SML922	LT.SV922	0.946			
Potassium			Potassium			Potassium		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.281	LT.AM922	LT.DL922	0.979	LT.AM422	LT.AM922	0.229
LT.AM422	LT.SML422	0.876	LT.AM922	LT.SML922	0.335	LT.DL422	LT.DL922	0.0355
LT.AM422	LT.SV422	0.169	LT.AM922	LT.SV922	0.00348	LT.SML422	LT.SML922	0.025
LT.DL422	LT.SML422	0.102	LT.DL922	LT.SML922	0.204	LT.SV422	LT.SV922	0.884
LT.DL422	LT.SV422	0.98	LT.DL922	LT.SV922	0.00555			
LT.SML422	LT.SV422	0.0596	LT.SML922	LT.SV922	0.000491			
Carbonate			Carbonate			Carbonate		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.999	LT.AM922	LT.DL922	0.348	LT.AM422	LT.AM922	0.169
LT.AM422	LT.SML422	1	LT.AM922	LT.SML922	0.0641	LT.DL422	LT.DL922	0.0468
LT.AM422	LT.SV422	0.894	LT.AM922	LT.SV922	0.0187	LT.SML422	LT.SML922	0.49
LT.DL422	LT.SML422	0.999	LT.DL922	LT.SML922	0.00587	LT.SV422	LT.SV922	0.875
LT.DL422	LT.SV422	0.94	LT.DL922	LT.SV922	0.00205			
LT.SML422	LT.SV422	0.903	LT.SML922	LT.SV922	0.812			
Sulfate			Sulfate			Sulfate		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.000817	LT.AM922	LT.DL922	0.137	LT.AM422	LT.AM922	0.345
LT.AM422	LT.SML422	0.96	LT.AM922	LT.SML922	0.203	LT.DL422	LT.DL922	0.00359
LT.AM422	LT.SV422	1	LT.AM922	LT.SV922	0.784	LT.SML422	LT.SML922	0.0348
LT.DL422	LT.SML422	0.0005	LT.DL922	LT.SML922	0.991	LT.SV422	LT.SV922	0.615
LT.DL422	LT.SV422	0.00088	LT.DL922	LT.SV922	0.457			
LT.SML422	LT.SV422	0.942	LT.SML922	LT.SV922	0.611			

Lotus tenuis; One-ANOVA – Tukey's HSD; compared are all soil properties. On the left side are spatial comparisons, all 4 sites across both seasons. On left site are seasonal comparisons, same site compared across both seasons. Always sample groups, a factorial combination of season and site, were compared.

422 = April; 922 = September ; Site : AM = Alte Muehle, DL = Darscholacke, SML = Sechsmahdlacke, SV = Seevorgelaende

Table S10 Spatial comparison and summary statistics of analyzed soil properties

Lotus tenuis, spatial comparison. Soil properties summary statistics, containing number of replicates (N), mean and standard deviation (mean(sd)) and Tukey HSD compact letter display, depicting a significant group across sites in both seasons.

	Site	Alte Muehle	Darscholacke	Sechsmahdlacke	Seevorgelaende	Alte Muehle	Darscholacke	Sechsmahdlacke	Seevorgelaende
	Season	April				September			
	N	3	3	3	3	3	3	3	3
SWC	mean(sd)	0.12 (0.002)	0.13 (0.026)	0.07 (0.005)	0.12 (0.088)	0.17 (0.075)	0.11 (0.039)	0.15 (0.066)	0.17 (0.011)
	Tukey	a	a	a	a	a	a	a	a
pH H2O	mean(sd)	8.97 (0.107)	8.72 (0.288)	8.85 (0.115)	8.99 (0.129)	8.78 (0.139)	8.67 (0.067)	8.82 (0.125)	8.75 (0.121)
	Tukey	a	a	a	a	a	a	a	a
pH CaCl2	mean(sd)	7.92 (0.168)	8.08 (0.15)	7.7 (0.035)	7.89 (0.229)	7.94 (0.06)	8.27 (0.154)	8.18 (0.192)	7.88 (0.13)
	Tukey	a	a	a	a	ab	a	ab	b
cond	mean(sd)	1692.13 (1371.95)	5688 (1904.309)	1483.67 (131.089)	883.07 (224.249)	862.47 (234.966)	1249.4 (750.676)	1991.33 (394.22)	1780.67 (272.148)
	Tukey	b	a	b	b	a	a	a	a
Na	mean(sd)	15.07 (10.835)	36.19 (5.787)	12.56 (1.391)	16.93 (16.2)	6.16 (3.897)	6.76 (4.845)	17.45 (3.335)	14.83 (2.206)
	Tukey	a	a	a	a	b	b	a	ab
Mg	mean(sd)	0.31 (0.041)	5.29 (2.173)	0.3 (0.039)	0.48 (0.275)	0.53 (0.471)	1.37 (0.734)	0.25 (0.111)	0.4 (0.082)
	Tukey	b	a	b	b	a	a	a	a
K	mean(sd)	0.29 (0.112)	0.5 (0.136)	0.22 (0.039)	0.54 (0.183)	0.19 (0.056)	0.22 (0.079)	0.08 (0.056)	0.52 (0.105)
	Tukey	a	a	a	a	b	b	b	a
Ca	mean(sd)	0.26 (0.035)	1.88 (1.025)	0.32 (0.051)	0.31 (0.069)	0.5 (0.285)	0.76 (0.312)	0.21 (0.04)	0.31 (0.055)
	Tukey	b	a	b	b	a	a	a	a
Cl	mean(sd)	2.39 (3.266)	8.71 (2.661)	1.83 (0.413)	1.73 (1.598)	0.61 (0.246)	1.15 (1.034)	2.83 (1.17)	2.3 (1.058)
	Tukey	b	a	b	b	a	a	a	a
CO3	mean(sd)	5.16 (1.091)	5.44 (1.464)	5.21 (0.69)	6.61 (4.646)	3.69 (1.069)	2.56 (0.971)	5.6 (0.542)	6.16 (0.125)
	Tukey	a	a	a	a	bc	c	ab	a
SO4	mean(sd)	1.9 (2.737)	15.72 (3.136)	0.87 (0.014)	2.05 (3.075)	0.21 (0.064)	2.48 (2.04)	2.22 (0.74)	1.07 (0.522)
	Tukey	b	a	b	b	a	a	a	a

Table S11 Seasonal comparison and summary statistics of analyzed soil properties

Lotus tenuis, seasonal comparison. Soil properties summary statistics, containing number of replicates (N), mean and standard deviation (mean(sd)) and Tukey HSD compact letter display, depicting a significant group across seasons at a particular site.

	Site	Alte Muehle		Darscholacke		Sechsmahdlacke		Seevorgelaende	
	Season	April	September	April	September	April	September	April	September
	N	3	3	3	3	3	3	3	3
SWC	mean(sd)	0.12 (0.002)	0.17 (0.075)	0.13 (0.026)	0.11 (0.039)	0.07 (0.005)	0.15 (0.066)	0.12 (0.088)	0.17 (0.011)
	Tukey	a	a	a	a	a	a	a	a
pH H2O	mean(sd)	8.97 (0.107)	8.78 (0.139)	8.72 (0.288)	8.67 (0.067)	8.85 (0.115)	8.82 (0.125)	8.99 (0.129)	8.75 (0.121)
	Tukey	a	a	a	a	a	a	a	a
pH CaCl2	mean(sd)	7.92 (0.168)	7.94 (0.06)	8.08 (0.15)	8.27 (0.154)	7.7 (0.035)	8.18 (0.192)	7.89 (0.229)	7.88 (0.13)
	Tukey	a	a	a	a	b	a	a	a
cond	mean(sd)	1692.13 (1371.95)	862.47 (234.966)	5688 (1904.309)	1249.4 (750.676)	1483.67 (131.089)	1991.33 (394.22)	883.07 (224.249)	1780.67 (272.148)
	Tukey	a	a	a	b	a	a	b	a
Na	mean(sd)	15.07 (10.835)	6.16 (3.897)	36.19 (5.787)	6.76 (4.845)	12.56 (1.391)	17.45 (3.335)	16.93 (16.2)	14.83 (2.206)
	Tukey	a	a	a	b	a	a	a	a
Mg	mean(sd)	0.31 (0.041)	0.53 (0.471)	5.29 (2.173)	1.37 (0.734)	0.3 (0.039)	0.25 (0.111)	0.48 (0.275)	0.4 (0.082)
	Tukey	a	a	a	b	a	a	a	a
K	mean(sd)	0.29 (0.112)	0.19 (0.056)	0.5 (0.136)	0.22 (0.079)	0.22 (0.039)	0.08 (0.056)	0.54 (0.183)	0.52 (0.105)
	Tukey	a	a	a	b	a	b	a	a
Ca	mean(sd)	0.26 (0.035)	0.5 (0.285)	1.88 (1.025)	0.76 (0.312)	0.32 (0.051)	0.21 (0.04)	0.31 (0.069)	0.31 (0.055)
	Tukey	a	a	a	a	a	a	a	a
Cl	mean(sd)	2.39 (3.266)	0.61 (0.246)	8.71 (2.661)	1.15 (1.034)	1.83 (0.413)	2.83 (1.17)	1.73 (1.598)	2.3 (1.058)
	Tukey	a	a	a	b	a	a	a	a
CO3	mean(sd)	5.16 (1.091)	3.69 (1.069)	5.44 (1.464)	2.56 (0.971)	5.21 (0.69)	5.6 (0.542)	6.61 (4.646)	6.16 (0.125)
	Tukey	a	a	a	b	a	a	a	a
SO4	mean(sd)	1.9 (2.737)	0.21 (0.064)	15.72 (3.136)	2.48 (2.04)	0.87 (0.014)	2.22 (0.74)	2.05 (3.075)	1.07 (0.522)
	Tukey	a	a	a	b	b	a	a	a

Table S12 Ratio of soil ions

L. tenuis, ratios of analyses soil ions across sites and seasons. Most abundant cation if sodium, on anion side it is predominantly carbonate, however at Darscholacke site is sulfate more abundant

Site	Alte Muehle		Darscholacke		Sechsmahdlacke		Seevorgelaende	
Season	April	September	April	September	April	September	April	September
Soil Properties	Mean							
Sodium[3]	15.07	6.16	36.19	6.76	12.56	17.45	16.93	14.83
Magnesium[3]	0.31	0.53	5.29	1.37	0.3	0.25	0.48	0.4
Potassium[3]	0.29	0.19	0.5	0.22	0.22	0.08	0.54	0.52
Calcium[3]	0.26	0.5	1.88	0.76	0.32	0.21	0.31	0.31
Chloride[3]	2.39	0.61	8.71	1.15	1.83	2.83	1.73	2.3
Carbonate[3]	5.16	3.69	5.44	2.56	5.21	5.6	6.61	6.16
Sulfate[3]	1.9	0.21	15.72	2.48	0.87	2.22	2.05	1.07

[3][mM/L]

Spatial comparison			Spatial comparison			Seasonal comparison		
April			September					
Chlorophyll a			**Chlorophyll a**			**Chlorophyll a**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.787	LT.AM922	LT.DL922	0.276	LT.AM422	LT.AM922	2.75E-05
LT.AM422	LT.SML422	0.0000385	LT.AM922	LT.SML922	0.236	LT.DL422	LT.DL922	4.56E-05
LT.AM422	LT.SV422	0.000000802	LT.AM922	LT.SV922	0.0186	LT.SML422	LT.SML922	0.61
LT.DL422	LT.SML422	0.000641	LT.DL922	LT.SML922	1	LT.SV422	LT.SV922	0.807
LT.DL422	LT.SV422	0.0000138	LT.DL922	LT.SV922	0.561			
LT.SML422	LT.SV422	0.545	LT.SML922	LT.SV922	0.623			
Chlorophyll b			**Chlorophyll b**			**Chlorophyll b**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.886	LT.AM922	LT.DL922	0.19	LT.AM422	LT.AM922	0.00621
LT.AM422	LT.SML422	0.318	LT.AM922	LT.SML922	0.679	LT.DL422	LT.DL922	1.41E-05
LT.AM422	LT.SV422	0.000747	LT.AM922	LT.SV922	0.0511	LT.SML422	LT.SML922	0.206
LT.DL422	LT.SML422	0.0801	LT.DL922	LT.SML922	0.765	LT.SV422	LT.SV922	0.391
LT.DL422	LT.SV422	0.0000848	LT.DL922	LT.SV922	0.924			
LT.SML422	LT.SV422	0.0586	LT.SML922	LT.SV922	0.381			
Carotenoids			**Carotenoids**			**Carotenoids**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.625	LT.AM922	LT.DL922	0.127	LT.AM422	LT.AM922	6.79E-07
LT.AM422	LT.SML422	4.13E-08	LT.AM922	LT.SML922	0.185	LT.DL422	LT.DL922	1.87E-06
LT.AM422	LT.SV422	0.00004	LT.AM922	LT.SV922	0.0268	LT.SML422	LT.SML922	0.148
LT.DL422	LT.SML422	0.00000153	LT.DL922	LT.SML922	0.997	LT.SV422	LT.SV922	0.000078
LT.DL422	LT.SV422	0.00149	LT.DL922	LT.SV922	0.901			
LT.SML422	LT.SV422	0.0955	LT.SML922	LT.SV922	0.803			
Phenols			**Phenols**			**Phenols**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.992	LT.AM922	LT.DL922	0.545	LT.AM422	LT.AM922	0.287
LT.AM422	LT.SML422	0.886	LT.AM922	LT.SML922	0.978	LT.DL422	LT.DL922	0.0891
LT.AM422	LT.SV422	0.383	LT.AM922	LT.SV922	0.999	LT.SML422	LT.SML922	0.993
LT.DL422	LT.SML422	0.971	LT.DL922	LT.SML922	0.293	LT.SV422	LT.SV922	0.393
LT.DL422	LT.SV422	0.551	LT.DL922	LT.SV922	0.413			
LT.SML422	LT.SV422	0.797	LT.SML922	LT.SV922	0.991			
Flavonoids			**Flavonoids**			**Flavonoids**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.0000098	LT.AM922	LT.DL922	0.00268	LT.AM422	LT.AM922	0.00069
LT.AM422	LT.SML422	0.136	LT.AM922	LT.SML922	1	LT.DL422	LT.DL922	0.5
LT.AM422	LT.SV422	0.29	LT.AM922	LT.SV922	0.894	LT.SML422	LT.SML922	0.725
LT.DL422	LT.SML422	0.00407	LT.DL922	LT.SML922	0.00147	LT.SV422	LT.SV922	0.0223
LT.DL422	LT.SV422	0.00125	LT.DL922	LT.SV922	0.00898			
LT.SML422	LT.SV422	0.972	LT.SML922	LT.SV922	0.843			
Soluble Sugars			**Soluble Sugars**			**Soluble Sugars**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.995	LT.AM922	LT.DL922	0.628	LT.AM422	LT.AM922	0.641
LT.AM422	LT.SML422	0.143	LT.AM922	LT.SML922	0.349	LT.DL422	LT.DL922	0.203
LT.AM422	LT.SV422	0.0921	LT.AM922	LT.SV922	0.987	LT.SML422	LT.SML922	0.000867
LT.DL422	LT.SML422	0.227	LT.DL922	LT.SML922	0.959	LT.SV422	LT.SV922	0.00177
LT.DL422	LT.SV422	0.153	LT.DL922	LT.SV922	0.377			
LT.SML422	LT.SV422	0.996	LT.SML922	LT.SV922	0.163			
Free Amino Acids			**Free Amino Acids**			**Free Amino Acids**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.609	LT.AM922	LT.DL922	0.92	LT.AM422	LT.AM922	0.362
LT.AM422	LT.SML422	0.000528	LT.AM922	LT.SML922	0.66	LT.DL422	LT.DL922	0.0673
LT.AM422	LT.SV422	0.00000961	LT.AM922	LT.SV922	0.995	LT.SML422	LT.SML922	9.54E-05
LT.DL422	LT.SML422	0.0000125	LT.DL922	LT.SML922	0.949	LT.SV422	LT.SV922	0.0101
LT.DL422	LT.SV422	0.000000228	LT.DL922	LT.SV922	0.788			
LT.SML422	LT.SV422	0.511	LT.SML922	LT.SV922	0.459			
Proline			**Proline**			**Proline**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.104	LT.AM922	LT.DL922	0.462	LT.AM422	LT.AM922	0.177
LT.AM422	LT.SML422	0.00000204	LT.AM922	LT.SML922	1	LT.DL422	LT.DL922	0.0305
LT.AM422	LT.SV422	0.00635	LT.AM922	LT.SV922	0.0768	LT.SML422	LT.SML922	7.7E-08
LT.DL422	LT.SML422	2.01E-09	LT.DL922	LT.SML922	0.409	LT.SV422	LT.SV922	0.317
LT.DL422	LT.SV422	0.00000571	LT.DL922	LT.SV922	0.724			
LT.SML422	LT.SV422	0.0357	LT.SML922	LT.SV922	0.0575			
Proteins			**Proteins**			**Proteins**		
group1	group2	p.adj	group1	group2	p.adj	group1	group2	p.adj
LT.AM422	LT.DL422	0.00219	LT.AM922	LT.DL922	0.758	LT.AM422	LT.AM922	0.0125
LT.AM422	LT.SML422	0.951	LT.AM922	LT.SML922	0.93	LT.DL422	LT.DL922	2.87E-08
LT.AM422	LT.SV422	1	LT.AM922	LT.SV922	0.999	LT.SML422	LT.SML922	6.02E-07
LT.DL422	LT.SML422	0.00036	LT.DL922	LT.SML922	0.979	LT.SV422	LT.SV922	0.00114
LT.DL422	LT.SV422	0.00155	LT.DL922	LT.SV922	0.635			
LT.SML422	LT.SV422	0.953	LT.SML922	LT.SV922	0.86			

Table S13 Metabolites and total proteins Tukey HSD p-values

Lotus tenuis, results of Tukey HSD comparison, of metabolites from Neusiedler See. On the left side are spatial comparisons, all 4 sites across both seasons. On left site are seasonal comparisons, same site compared across both seasons. Always sample groups, a factorial combination of season and site, were compared.

422 = April; 922 = September ; Site : AM = Alte Muehle, DL = Darscholacke, SML = Sechsmahdlacke, SV = Seevorgelaende

Table S14 Control comparison, Dunnett's test p-values

Lotus tenuis and *Lotus japonicus* controls compared to each other and *Lotus tenuis* from Neusiedler See across sites and seasons. Represented are p-values of Dunnett tests. Overall were 4 post hoc comparisons conducted, all data to *Lotus tenuis* N-fix, N-fed, *Lotus japonicus* N-fix and N-fed. Note that same comparisons in different runs may produce slightly different p-values, but only at the later decimal places. Also, all comparisons were done from one data frame and later were results extracted for plots.

	***Lotus tenuis* N-fix**								
	Chlorophyll a	**Chlorophyll b**	**Carotenoids**	**Phenols**	**Flavonoids**	**Soluble Sugars**	**Free Amino Acids**	**Proline**	**Proteins**
LT.AM422	5.879E-06	3.398E-03	2.251E-06	8.096E-03	1.000E+00	5.931E-03	1.258E-01	2.819E-02	8.335E-01
LT.AM922	9.531E-01	8.436E-01	9.969E-01	3.386E-04	2.338E-02	4.462E-02	8.528E-01	8.401E-04	3.019E-02
LT.DL422	3.808E-04	1.067E-04	1.865E-04	2.909E-03	1.256E-10	1.999E-03	6.194E-03	2.406E-05	1.896E-03
LT.DL922	9.976E-01	1.000E+00	3.490E-02	7.116E-07	1.204E-07	2.553E-01	2.932E-01	1.167E-01	2.141E-04
LT.SML422	1.000E+00	6.009E-01	8.157E-01	3.585E-04	3.389E-02	3.209E-08	6.167E-01	1.370E-01	9.733E-01
LT.SML922	9.935E-01	9.993E-01	5.878E-02	8.909E-04	2.070E-02	4.611E-01	7.550E-02	4.418E-04	1.624E-03
LT.SV422	5.952E-01	3.975E-01	9.628E-01	6.865E-06	1.338E-01	4.050E-09	5.961E-02	1.000E+00	8.248E-01
LT.SV922	4.325E-01	1.000E+00	3.815E-03	1.857E-04	2.331E-03	1.362E-02	9.547E-01	7.820E-01	3.025E-02
LT.Nfed	6.973E-02	3.890E-02	2.794E-01	5.553E-03	1.302E-01	2.582E-01	4.978E-03	4.781E-03	8.511E-01
LJ.Nfix	1.104E-06	2.241E-04	4.152E-08	5.773E-02	1.970E-01	9.682E-01	9.446E-04	2.755E-04	2.653E-01
LJ.Nfed	2.872E-02	2.352E-02	2.065E-02	7.389E-03	1.565E-02	9.975E-01	6.425E-01	2.262E-01	9.703E-01
	***Lotus tenuis* N-fed**								
	Chlorophyll a	**Chlorophyll b**	**Carotenoids**	**Phenols**	**Flavonoids**	**Soluble Sugars**	**Free Amino Acids**	**Proline**	**Proteins**
LT.AM422	8.190E-02	9.919E-01	5.546E-03	8.703E-10	1.067E-01	1.806E-06	7.832E-01	9.941E-01	1.000E+00
LT.AM922	5.283E-01	5.412E-01	8.127E-02	2.327E-11	6.004E-06	6.819E-05	1.619E-01	9.980E-01	8.373E-04
LT.DL422	5.414E-01	4.855E-01	1.162E-01	3.211E-09	5.551E-16	3.776E-07	1.000E+00	7.377E-01	6.535E-02
LT.DL922	1.249E-02	3.703E-02	3.953E-05	1.987E-14	8.743E-13	8.018E-04	5.501E-01	8.551E-01	1.509E-06
LT.SML422	3.219E-02	6.057E-01	1.032E-02	5.950E-12	5.506E-06	1.901E-13	7.400E-06	1.809E-07	1.000E+00
LT.SML922	9.722E-03	1.464E-01	8.254E-05	5.761E-11	2.736E-06	2.704E-03	9.504E-01	9.952E-01	1.984E-05
LT.SV422	3.636E-04	5.756E-05	8.264E-01	6.117E-14	4.291E-05	7.505E-14	2.553E-08	2.665E-03	1.000E+00
LT.SV922	2.111E-04	1.045E-02	1.053E-06	8.614E-12	1.151E-07	8.865E-06	5.642E-02	1.193E-01	6.615E-04
LT.Nfix	6.984E-02	3.905E-02	2.794E-01	5.640E-03	1.301E-01	2.581E-01	5.135E-03	4.687E-03	8.511E-01
LJ.Nfix	1.642E-02	4.936E-01	1.957E-04	9.947E-01	1.000E+00	8.916E-01	9.981E-01	9.562E-01	9.630E-01
LJ.Nfed	9.994E-01	1.000E+00	8.035E-01	1.000E+00	9.356E-01	7.884E-01	3.755E-01	8.264E-01	1.000E+00
	***Lotus japonicus* N-fix**								
	Chlorophyll a	**Chlorophyll b**	**Carotenoids**	**Phenols**	**Flavonoids**	**Soluble Sugars**	**Free Amino Acids**	**Proline**	**Proteins**
LT.AM422	9.841E-01	9.207E-01	7.894E-01	6.858E-07	1.603E-01	3.982E-04	3.493E-01	4.945E-01	9.467E-01
LT.AM922	1.240E-04	1.689E-02	3.487E-09	8.343E-09	1.643E-05	4.502E-03	4.547E-02	1.000E+00	4.230E-05
LT.DL422	4.295E-01	1.000E+00	1.486E-01	5.973E-08	5.440E-15	1.203E-04	9.827E-01	1.000E+00	4.840E-01
LT.DL922	7.902E-08	2.141E-04	4.075E-14	8.393E-13	2.911E-12	3.769E-02	2.054E-01	2.443E-01	3.683E-08
LT.SML422	8.813E-08	1.502E-02	5.018E-12	1.219E-09	1.254E-05	8.174E-10	1.126E-06	2.496E-09	7.379E-01
LT.SML922	6.736E-08	1.596E-03	8.138E-14	4.191E-08	1.923E-05	8.697E-02	5.706E-01	1.000E+00	4.991E-07
LT.SV422	1.199E-10	3.224E-08	4.723E-07	2.043E-11	1.383E-04	2.043E-10	4.542E-09	1.376E-04	9.336E-01
LT.SV922	6.063E-11	4.458E-05	2.220E-16	2.288E-09	6.797E-07	9.876E-04	1.256E-02	1.152E-02	3.195E-05
LT.Nfix	9.027E-07	2.401E-04	2.605E-08	5.520E-02	1.878E-01	9.619E-01	9.673E-04	2.513E-04	2.531E-01
LT.Nfed	1.583E-02	4.731E-01	1.668E-04	9.933E-01	1.000E+00	8.767E-01	9.976E-01	9.482E-01	9.560E-01
LJ.Nfed	1.087E-01	8.375E-01	2.639E-02	9.781E-01	8.887E-01	1.000E+00	1.326E-01	2.510E-01	9.034E-01
	***Lotus japonicus* N-fed**								
	Chlorophyll a	**Chlorophyll b**	**Carotenoids**	**Phenols**	**Flavonoids**	**Soluble Sugars**	**Free Amino Acids**	**Proline**	**Proteins**
LT.AM422	3.415E-01	1.000E+00	2.603E-01	6.122E-09	1.073E-02	1.563E-03	9.892E-01	9.974E-01	1.000E+00
LT.AM922	2.444E-01	3.193E-01	4.099E-03	1.933E-10	6.489E-07	1.275E-02	1.000E+00	3.951E-01	4.139E-03
LT.DL422	9.390E-01	8.306E-01	9.214E-01	1.124E-09	3.331E-16	5.994E-04	4.045E-01	9.608E-02	5.898E-02
LT.DL922	4.581E-03	2.044E-02	9.242E-07	3.318E-13	7.960E-14	8.271E-02	1.000E+00	1.000E+00	1.856E-05
LT.SML422	1.202E-02	3.579E-01	2.770E-04	8.308E-11	3.760E-07	5.098E-09	3.253E-02	2.445E-04	1.000E+00
LT.SML922	3.631E-03	7.891E-02	1.948E-06	4.778E-10	1.346E-07	1.672E-01	9.262E-01	3.371E-01	1.979E-04
LT.SV422	1.567E-04	3.981E-05	1.102E-01	3.345E-13	3.691E-06	1.238E-09	9.824E-04	1.760E-01	1.000E+00
LT.SV922	8.763E-05	6.107E-03	1.802E-08	3.381E-11	1.084E-08	3.693E-03	9.954E-01	9.096E-01	3.759E-03
LT.Nfix	2.642E-02	2.119E-02	1.913E-02	6.893E-03	1.442E-02	9.956E-01	5.916E-01	2.034E-01	9.557E-01
LT.Nfed	9.989E-01	9.999E-01	7.571E-01	1.000E+00	9.102E-01	7.410E-01	3.386E-01	7.823E-01	1.000E+00
LJ.Nfix	1.025E-01	8.143E-01	2.532E-02	9.721E-01	8.697E-01	1.000E+00	1.252E-01	2.366E-01	8.859E-01

	Chlorophyll a			**Chlorophyll b**			**Carotenoids**		
Sample.group	**mean**	**n**	**sd**	**mean**	**n**	**sd**	**mean**	**n**	**sd**
LT.Nfix	37.52996	8	10.37879	10.63135	8	2.599454	9.369917	8	2.536196
LT.Nfed	28.66666	8	5.808613	7.540602	8	1.343578	7.817327	8	1.378056
LJ.Nfix	17.64317	7	3.735696	5.690104	7	1.039826	4.195356	7	1.102687
LJ.Nfed	26.73563	6	4.157148	7.078217	6	0.928808	6.778643	6	1.174733
	Phenols			**Flavonoids**			**Soluble Sugars**		
Sample.group	**mean**	**n**	**sd**	**mean**	**n**	**sd**	**mean**	**n**	**sd**
LT.Nfix	20.12094	8	2.892704	50.82292	8	6.553024	224.9817	8	49.93258
LT.Nfed	15.31136	8	2.207619	36.73403	8	9.322703	163.7148	8	25.76444
LJ.Nfix	16.28616	7	1.660049	37.43547	7	7.210393	197.5579	7	20.17082
LJ.Nfed	15.05354	6	0.618563	30.38645	6	2.825016	204.5725	6	28.12836
	Amino Acids			**Proline**			**Proteins**		
Sample.group	**mean**	**n**	**sd**	**mean**	**n**	**sd**	**mean**	**n**	**sd**
LT.Nfix	92.98913	8	21.50377	531.0836	8	114.4772	4.91761	8	2.278483
LT.Nfed	50.87329	8	20.23143	288.7442	8	143.5321	3.864077	8	0.938233
LJ.Nfix	43.41992	7	9.57732	223.6715	7	60.97916	3.016841	7	1.37344
LJ.Nfed	74.31915	6	25.54514	376.7465	6	150.4307	4.263107	6	0.995392

Table S15 Summary statistics of Lotus control plants

Table contains mean, number of replicates (n) and standard deviation (sd). *LT = Lotus tenuis; LJ = Lotus japonicus; Nfed = nitrogen fed; Nfix = nitrogen fixing*

Lepidium summary tables, one-way ANOVA, Tukey HSD & Dunnett's Test

Spatial comparison				Seasonal comparison		
SWC				**SWC**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.573	LC.AM422	LC.AM922	0.446
September	LC.AM922	LC.SV922	0.887	LC.SV422	LC.SV922	0.884
pH H2O				**pH H2O**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.0017	LC.AM422	LC.AM922	0.227
September	LC.AM922	LC.SV922	0.0301	LC.SV422	LC.SV922	0.406
Conductivity				**Conductivity**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.538	LC.AM422	LC.AM922	0.243
September	LC.AM922	LC.SV922	0.146	LC.SV422	LC.SV922	0.563
pH CaCl2				**pH CaCl2**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.0465	LC.AM422	LC.AM922	0.184
September	LC.AM922	LC.SV922	0.846	LC.SV422	LC.SV922	0.0361
Magnesium				**Magnesium**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.0605	LC.AM422	LC.AM922	0.149
September	LC.AM922	LC.SV922	0.0624	LC.SV422	LC.SV922	0.512
Sodium				**Sodium**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.809	LC.AM422	LC.AM922	0.131
September	LC.AM922	LC.SV922	0.111	LC.SV422	LC.SV922	0.467
Chloride				**Chloride**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.00136	LC.AM422	LC.AM922	0.281
September	LC.AM922	LC.SV922	0.209	LC.SV422	LC.SV922	0.489
Calcium				**Calcium**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.00665	LC.AM422	LC.AM922	0.131
September	LC.AM922	LC.SV922	0.0537	LC.SV422	LC.SV922	0.181
Potassium				**Potassium**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.00093	LC.AM422	LC.AM922	0.401
September	LC.AM922	LC.SV922	0.00966	LC.SV422	LC.SV922	0.807
Carbonate				**Carbonate**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.0222	LC.AM422	LC.AM922	0.127
September	LC.AM922	LC.SV922	0.0198	LC.SV422	LC.SV922	0.551
Sulfate				**Sulfate**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.00538	LC.AM422	LC.AM922	0.18
September	LC.AM922	LC.SV922	0.675	LC.SV422	LC.SV922	0.336

Table S16 Soil properties Tukey HSD p-values *Lepidium cartilagineum*; compared are all soil properties. On the left side are spatial comparisons, all 4 sites across both seasons. On left site are seasonal comparisons, same site compared across both seasons. Always sample groups, a factorial combination of season and site, were compared.

422 = April; 922 = September ; Site : AM = Alte Muehle, SV = Seevorgelaende

Table S17 Spatial comparison and summary statistics of analyzed soil properties

Lepidium cartilagineum, soil properties summary statistics, containing number of replicates (N), mean and standard deviation (mean(sd)) and Tukey HSD compact letter display, depicting a significant group across sites in both seasons. (Spatial comparison)

	Site	Alte Muehle	Seevorgelaende	Alte Muehle	Seevorgelaende
	Season	April	April	September	September
	N	3	3	3	3
SWC	mean (sd)	0.142 (0.011)	0.156 (0.037)	0.157 (0.027)	0.164 (0.078)
	Tukey spatial	a	a	a	a
pH H2O	mean (sd)	9.217 (0.015)	9.55 (0.075)	8.962 (0.309)	9.667 (0.204)
	Tukey spatial	b	a	b	a
pH CaCl2	mean (sd)	8.387 (0.138)	8.123 (0.081)	8.773 (0.394)	8.84 (0.392)
	Tukey spatial	a	b	a	a
cond	mean (sd)	12903.333 (1466.47)	13873.333 (2020.528)	10442 (2745.154)	15510 (4022.387)
	Tukey spatial	a	a	a	a
Mg	mean (sd)	0.057 (0.012)	0.035 (0.009)	0.101 (0.041)	0.04 (0.007)
	Tukey spatial	a	a	a	a
SO4	mean (sd)	27.906 (3.576)	13.6 (2.759)	20.608 (6.916)	18.112 (6.595)
	Tukey spatial	a	b	a	a
Ca	mean (sd)	0.052 (0.01)	0.021 (0.002)	0.108 (0.05)	0.029 (0.008)
	Tukey spatial	a	b	a	a
Na	mean (sd)	139.667 (17.842)	144 (22.928)	101.07 (30.452)	169.263 (49.372)
	Tukey spatial	a	a	a	a
K	mean (sd)	0.945 (0.077)	2.088 (0.212)	0.838 (0.184)	2.02 (0.4)
	Tukey spatial	b	a	b	a
Cl	mean (sd)	25.58 (2.169)	14.612 (1.002)	21.779 (4.817)	16.4 (3.944)
	Tukey spatial	a	b	a	a
CO3	mean (sd)	29.719 (6.126)	52.195 (8.811)	19.665 (6.66)	59.398 (17.065)
	Tukey spatial	b	a	b	a

Table S18 Seasonal comparison and summary statistics of analyzed soil properties

Lepidium cartilagineum, soil properties summary statistics, containing number of replicates (N), mean and standard deviation (mean(sd)) and Tukey HSD compact letter display, depicting a significant group across sites in both seasons. (Seasonal comparison)

	Site	Alte Muhele	Alte Muehle	Seevorgelaende	Seevorgelaende
	Season	April	September	April	September
	N	3	3	3	3
SWC	mean (sd)	0.142 (0.011)	0.157 (0.027)	0.156 (0.037)	0.164 (0.078)
	Tukey season	a	a	a	a
pH H2O	mean (sd)	9.217 (0.015)	8.962 (0.309)	9.55 (0.075)	9.667 (0.204)
	Tukey season	a	a	a	a
pH CaCl2	mean (sd)	8.387 (0.138)	8.773 (0.394)	8.123 (0.081)	8.84 (0.392)
	Tukey season	a	a	b	a
cond	mean (sd)	12903.333 (1466.47)	10442 (2745.154)	13873.333 (2020.528)	15510 (4022.387)
	Tukey season	a	a	a	a
Mg	mean (sd)	0.057 (0.012)	0.101 (0.041)	0.035 (0.009)	0.04 (0.007)
	Tukey season	a	a	a	a
SO4	mean (sd)	27.906 (3.576)	20.608 (6.916)	13.6 (2.759)	18.112 (6.595)
	Tukey season	a	a	a	a
Ca	mean (sd)	0.052 (0.01)	0.108 (0.05)	0.021 (0.002)	0.029 (0.008)
	Tukey season	a	a	a	a
Na	mean (sd)	139.667 (17.842)	101.07 (30.452)	144 (22.928)	169.263 (49.372)
	Tukey season	a	a	a	a
K	mean (sd)	0.945 (0.077)	0.838 (0.184)	2.088 (0.212)	2.02 (0.4)
	Tukey season	a	a	a	a
Cl	mean (sd)	25.58 (2.169)	21.779 (4.817)	14.612 (1.002)	16.4 (3.944)
	Tukey season	a	a	a	a
CO3	mean (sd)	29.719 (6.126)	19.665 (6.66)	52.195 (8.811)	59.398 (17.065)
	Tukey season	a	a	a	a

Table S19 Ratio of soil ions

L. cartilagineum, ratios of analyses soil ions across sites and seasons. Most abundant cation if sodium, on anion side it is predominantly carbonate at Seevorgelände. At Alte Mühle, chloride, carbonate and sulfate had similar content.

Site	Alte Muhle		Seevorgelaende	
Season	April	September	April	September
Soil Properties	Mean and (Standard deviation)		TukeyHSD seasonally, spatially	
Sodium[3]	139.667	101.07	144	169.263
Magnesium[3]	0.057	0.101	0.035	0.04
Potassium[3]	0.945	0.838	2.088	2.02
Calcium[3]	0.052	0.108	0.021	0.029
Chloride[3]	25.58	21.779	14.612	16.4
Carbonate[3]	29.719	19.665	52.195	59.398
Sulfate[3]	27.906	20.608	13.6	18.112

[3][mM/L]

Table S20 Metabolites and total proteins Tukey HSD p-values

Lepidium cartilagineum, results of Tukey HSD comparison, of metabolites from Neusiedler See. On the left side are spatial comparisons, both sites across both seasons. On left site are seasonal comparisons, same site compared across both seasons. Always sample groups, a factorial combination of season and site, were compared.

	Spatial comparison			Seasonal comparison		
	Chlorophyll a			**Chlorophyll a**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	3.15E-06	LC.AM422	LC.AM922	9.35E-08
September	LC.AM922	LC.SV922	0.15	LC.SV422	LC.SV922	0.196
	Chlorophyll b			**Chlorophyll b**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.000162	LC.AM422	LC.AM922	0.00000101
September	LC.AM922	LC.SV922	0.126	LC.SV422	LC.SV922	0.00324
	Carotenoids			**Carotenoids**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	1.37E-06	LC.AM422	LC.AM922	6.88E-09
September	LC.AM922	LC.SV922	0.0383	LC.SV422	LC.SV922	0.00731
	Flavonoids			**Flavonoids**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.000243	LC.AM422	LC.AM922	0.491
September	LC.AM922	LC.SV922	0.173	LC.SV422	LC.SV922	0.0737
	Phenols			**Phenols**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.00167	LC.AM422	LC.AM922	0.00000241
September	LC.AM922	LC.SV922	0.103	LC.SV422	LC.SV922	0.00714
	Soluble Sugars			**Soluble Sugars**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.697	LC.AM422	LC.AM922	0.00358
September	LC.AM922	LC.SV922	0.000113	LC.SV422	LC.SV922	0.00000503
	Free Amino Acids			**Free Amino Acids**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.388	LC.AM422	LC.AM922	0.846
September	LC.AM922	LC.SV922	0.47	LC.SV422	LC.SV922	0.448
	Proline			**Proline**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.465	LC.AM422	LC.AM922	0.488
September	LC.AM922	LC.SV922	0.364	LC.SV422	LC.SV922	0.486
	Proteins			**Proteins**		
	group1	group2	p.adj	group1	group2	p.adj
April	LC.AM422	LC.SV422	0.396	LC.AM422	LC.AM922	0.00764
September	LC.AM922	LC.SV922	0.974	LC.SV422	LC.SV922	0.0112

422 = April; 922 = September ; Site : AM = Alte Mühle, SV = Seevorgelaende

Table S21 Control comparison, Dunnett's test p-values

Lepidium cartilagineum and *Lepidium sativum* controls compared to each other and *Lepidium cartilagineum* from Neusiedler See across sites and seasons. Represented are p-values of Dunnett tests. Overall were 2 post hoc comparisons conducted, all data to *Lepidium cartilagineum* and *Lepidium sativum*. Note that same comparisons in different runs may produce slightly different p-values, but only at the later decimal places. Also, all comparisons were done from one data frame and later were results extracted for plots.

	***Lepidium cartilagineum* control**								
	Chlorophyll a	**Chlorophyll b**	**Carotenoids**	**Phenols**	**Flavonoids**	**Soluble Sugars**	**Free Amino Acids**	**Proline**	**Proteins**
LC.AM422	1.11022E-15	1.19904E-14	5.78981E-13	9.97383E-05	0.315768707	3.34177E-14	0.999998477	0.929467039	0.502005419
LC.AM922	1.56301E-07	1.81575E-05	0.141798874	1.52034E-12	0.103685389	4.07898E-07	0.999870389	0.999843291	0.253537233
LC.SV422	1.87593E-07	3.86396E-07	0.008001297	5.41523E-09	3.61456E-06	2.8999E-13	0.628891867	0.998587172	0.578702074
LC.SV922	3.49492E-05	0.004941082	0.998558717	2.22045E-16	0.002394712	0.020217593	0.985217948	0.803219109	0.231228994
LS.GC	5.38458E-14	3.93308E-12	1.4222E-13	1.48726E-06	0.974156801	0.737232065	9.59394E-09	1.84137E-08	0.844108659
	Lepidium sativum								
	Chlorophyll a	**Chlorophyll b**	**Carotenoids**	**Phenols**	**Flavonoids**	**Soluble Sugars**	**Free Amino Acids**	**Proline**	**Proteins**
LC.AM422	0.998111586	0.968945723	0.624028112	1.22125E-15	0.707861921	1.44329E-15	2.14097E-09	5.10816E-10	0.990833086
LC.AM922	0.000380241	0.000233276	2.1946E-11	0	0.336690229	3.04559E-09	1.47963E-09	1.17367E-08	0.041149759
LC.SV422	0.000436784	0.041431225	1.02461E-08	0	5.03102E-05	7.74936E-14	3.82103E-07	4.21662E-08	0.997702261
LC.SV922	1.57973E-06	3.13676E-07	4.44089E-16	0	0.017221457	0.001278798	6.98506E-08	1.83945E-07	0.036707989
LC.GC	9.21485E-15	1.12004E-11	1.02807E-13	3.20929E-06	0.970946954	0.721228573	2.08759E-08	2.13403E-08	0.831515479

Table S22 Summary statistics of Lepidium control plants

Table contains mean, number of replicates (n) and standard deviation (sd). *LC = Lepidium cartilagineum; LS= Lepidium sativum; GC = growth chamber*

	Chlorophyll A			**Chlorophyll B**			**Carotenoids**		
Sample.group	**mean**	**n**	**sd**	**mean**	**n**	**sd**	**mean**	**n**	**sd**
LC.GC	48.88864	8	7.661447	12.293	8	1.812856	11.22648	8	1.035451
LS.GC	19.94829	7	5.056349	5.84321	7	1.206448	4.779399	7	1.123204
	Phenols			**Flavonoids**			**Soluble Sugars**		
Sample.group	**mean**	**n**	**sd**	**mean**	**n**	**sd**	**mean**	**n**	**sd**
LC.GC	23.04179	8	3.451063	64.65057	8	5.996903	179.5149	8	30.22767
LS.GC	12.33324	7	2.156314	67.25662	7	11.83059	156.8718	7	33.08931
	Free Amino Acids			**Proline**			**Proteins**		
Sample.group	mean	**n**	**sd**	**mean**	**n**	**sd**	**mean**	**n**	**sd**
LC.GC	546.0353	8	58.43946	3184.189	8	379.8104	8.391696	8	3.376123
LS.GC	43.93464	7	11.00026	217.6757	7	62.88772	4.199907	7	3.368352

Raw data used for analysis

Table S23 Metabolites data used for RDA analysis, *Lotus tenuis*

Metabolites of first 3 replicates – concentrations, used only for RDA analysis. Due to loss of first replicate of *Lotus tenuis* from September at AM, the metabolites concentrations were imputed using mean concentrations of the rest replicates (replicates; n = 5).). Concentrations are in µg/mg of dried weight (pigments, phenols, flavonoids, proteins); nmol/mg of dried weight (soluble sugars) and µmol/mg of dried weight (free amino acids, proline). *ChlA = chlorophyll a, ChlB = chlorophyll b, Carot = carotenoids, Phe = phenols, Flav = flavonoids, SS = soluble sugars, AA = free amino acids, Pro = proline, Prot = proteins*

	name	Season	Site	Species	ST	Species.Season	Sample.group	ChlA	ChlB	Carot	Phe	Flav	AA	Pro	SS	Prot
	LT_AM1	422	AlteMuehle	Lotus.tenuis	AM	LT.422	LT.AM422	25.33024	7.341084	6.133079	24.70855	61.06027	41.39748	151.4905	323.1812	2.759046
	LT_AM2	422	AlteMuehle	Lotus.tenuis	AM	LT.422	LT.AM422	19.14832	5.974552	4.874538	24.81233	52.42171	60.30937	271.9061	401.2113	3.841205
	LT_AM3	422	AlteMuehle	Lotus.tenuis	AM	LT.422	LT.AM422	29.87445	10.57276	7.59563	28.7869	62.00343	85.99821	457.8382	405.9404	4.877073
IMPUTED	**LT_AM1 I**	**922**	**AlteMuehle**	**Lotus.tenuis**	**AM**	**LT.922**	**LT.AM922**	**33.65215**	**9.426305**	**9.767791**	**27.89147**	**71.77203**	**88.22368**	**220.7932**	**327.2642**	**9.242806**
	LT_AM2 I	922	AlteMuehle	Lotus.tenuis	AM	LT.922	LT.AM922	32.73796	7.908387	9.509066	24.43439	68.63116	59.01297	374.5681	282.7104	3.587679
	LT_AM3 II	922	AlteMuehle	Lotus.tenuis	AM	LT.922	LT.AM922	38.85607	10.19016	10.86744	19.77895	59.4788	51.16979	241.9247	267.4995	4.030999
	LT_SV1	422	Seevorgelaende	Lotus.tenuis	SV	LT.422	LT.SV422	31.0393	8.666106	7.663601	24.24044	62.01706	114.8633	464.4448	377.748	1.49846
	LT_SV2	422	Seevorgelaende	Lotus.tenuis	SV	LT.422	LT.SV422	38.5377	8.222925	9.365085	29.69317	61.70354	145.761	630.9156	461.744	2.34451
	LT_SV3	422	Seevorgelaende	Lotus.tenuis	SV	LT.422	LT.SV422	56.31178	20.83105	6.030942	29.86869	64.80715	96.61094	385.5877	395.0842	3.083798
	LT_SV1 I	922	Seevorgelaende	Lotus.tenuis	SV	LT.922	LT.SV922	40.2751	10.73695	11.34515	24.71511	66.13614	89.9226	512.9388	278.3225	7.213163
	LT_SV2 I	922	Seevorgelaende	Lotus.tenuis	SV	LT.922	LT.SV922	45.82911	9.895224	13.35352	31.65433	94.25358	82.27884	440.9975	288.9551	5.773337
	LT_SV3 II	922	Seevorgelaende	Lotus.tenuis	SV	LT.922	LT.SV922	44.92867	10.93671	12.79434	24.63695	69.97056	46.50898	263.8102	299.6704	8.249665
	LT_DL1	422	Darscholacke	Lotus.tenuis	DL	LT.422	LT.DL422	20.76939	4.9483	5.025783	28.85716	82.68039	38.8664	138.1653	368.0546	0.363593
	LT_DL2	422	Darscholacke	Lotus.tenuis	DL	LT.422	LT.DL422	28.84936	7.458068	7.858452	26.25355	151.8218	78.94425	372.7672	554.1934	0.508814
	LT_DL3	422	Darscholacke	Lotus.tenuis	DL	LT.422	LT.DL422	32.21353	9.174519	8.660788	23.39684	90.1161	77.36376	319.8664	328.6991	2.142036
	LT_DL 1 II	922	Darscholacke	Lotus.tenuis	DL	LT.922	LT.DL922	41.068	10.61979	11.35747	30.82602	96.24578	55.41227	346.6786	281.8064	11.44543
	LT_DL2 I	922	Darscholacke	Lotus.tenuis	DL	LT.922	LT.DL922	47.60002	11.43531	13.60958	24.61544	84.00005	78.50105	448.6871	289.7386	7.222036
	LT_DL3 II	922	Darscholacke	Lotus.tenuis	DL	LT.922	LT.DL922	30.68288	8.895755	9.789417	29.22912	80.57736	84.76137	667.009	282.0046	11.69534
	LT_SML1 II	422	Sechsmahdlacke	Lotus.tenuis	SML	LT.422	LT.SML422	27.79578	7.44973	8.553229	22.37796	78.23005	90.02325	648.1798	302.8906	3.58276
	LT_SML2 II	422	Sechsmahdlacke	Lotus.tenuis	SML	LT.422	LT.SML422	31.55536	7.601419	9.16218	26.80297	58.98359	98.82256	663.6867	327.2793	4.024857
	LT_SML 3 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT.422	LT.SML422	33.18648	8.052928	9.080362	21.34491	55.5225	94.6228	623.3118	351.4731	4.956257
	LT_SML1 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT.922	LT.SML922	47.2892	12.58118	12.722	22.80566	68.27594	50.77184	200.841	286.8756	9.840655
	LT_SML 2 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT.922	LT.SML922	43.16323	9.90621	12.42528	25.51089	67.21995	59.72526	332.515	289.3846	8.152025
	LT_SML3 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT.922	LT.SML922	29.96264	8.569326	8.840702	28.45875	78.89173	56.49733	361.4914	244.4771	8.426341

Table S24 Soil data used for RDA, Tukey HSD multiple comparison, *Lotus tenuis*

Imputed were replicates only in metabolites. Concentrations are given in decimal percentages for soil water content; Ece (mS/cm) for soil salinity; pH for water and $CaCl_2$ and mM/L for all soil ions (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}, CO_3^{2-}).

name	Season	Site	Species	ST	Sample.group	pH_CaCl2	pH_H2O	SWC_BD	conductivity	magnesium_mM	calcium_mM	sodium_mM	potassium_mM	chloride_mM	sulfate_mM	carbonate_mM
LT_AM1	422	AlteMuehle	Lotus.tenuis	AM	LT.AM422	8.1	9.04	0.116999	3264	0.332297272	0.222887524	27.57294272	0.336592269	6.159713888	5.060492538	6.369602808
LT_AM2	422	AlteMuehle	Lotus.tenuis	AM	LT.AM422	7.77	9.03	0.119945	1077	0.339404959	0.27843116	9.271698818	0.376654225	0.462372846	0.332652398	4.878173821
LT_AM3	422	AlteMuehle	Lotus.tenuis	AM	LT.AM422	7.88	8.85	0.119209	735.4	0.265545955	0.286979001	8.373544915	0.16644034	0.542946894	0.307677671	4.243366466
LT_AM1 I	922	AlteMuehle	Lotus.tenuis	AM	LT.AM922	7.88	8.82	0.134257	618.3	1.066361002	0.815002196	2.071118347	0.128097742	0.330975506	0.141202871	2.674280619
LT_AM2 I	922	AlteMuehle	Lotus.tenuis	AM	LT.AM922	7.94	8.63	0.25679	882.1	0.338191503	0.421185049	6.562454176	0.236725847	0.711954651	0.224712087	3.578277152
LT_AM3 II	922	AlteMuehle	Lotus.tenuis	AM	LT.AM922	8	8.9	0.119574	1087	0.183797172	0.261289901	9.833915203	0.208078677	0.791615108	0.266383462	4.803892997
LT_SV1	422	Seevorgelaende	Lotus.tenuis	SV	LT.SV422	7.82	8.94	0.072007	1142	0.299469739	0.234016301	9.481498504	0.502322958	1.017773134	0.35892275	4.657587454
LT_SV2	422	Seevorgelaende	Lotus.tenuis	SV	LT.SV422	7.71	8.9	0.060326	755.3	0.35121116	0.323367833	5.787413424	0.375284578	0.618195411	0.195000416	3.251829873
LT_SV3	422	Seevorgelaende	Lotus.tenuis	SV	LT.SV422	8.15	9.14	0.218717	751.9	0.799493364	0.369314611	35.50993555	0.73666664	3.563663818	5.601198531	11.90907863
LT_SV1 I	922	Seevorgelaende	Lotus.tenuis	SV	LT.SV922	8.01	8.71	0.169293	2063	0.305529048	0.255914137	17.21508883	0.589893709	3.491184317	1.63098026	6.087362034
LT_SV2 I	922	Seevorgelaende	Lotus.tenuis	SV	LT.SV922	7.75	8.66	0.181655	1759	0.465023643	0.305815021	14.40838403	0.569489379	1.954998062	0.979825539	6.302450796
LT_SV3 II	922	Seevorgelaende	Lotus.tenuis	SV	LT.SV922	7.89	8.89	0.159939	1520	0.416891916	0.365629354	12.86409579	0.398094894	1.461625111	0.598611841	6.084192215
LT_DL1	422	Darscholacke	Lotus.tenuis	DL	LT.DL422	8.16	8.92	0.138002	3857	3.348152501	1.01988721	29.61922374	0.439191728	5.636602693	12.12515752	4.453788586
LT_DL2	422	Darscholacke	Lotus.tenuis	DL	LT.DL422	7.91	8.39	0.14903	7658	7.638225159	3.013991025	38.41316385	0.65362713	10.33114342	17.8960353	7.124004668
LT_DL3	422	Darscholacke	Lotus.tenuis	DL	LT.DL422	8.18	8.85	0.099362	5549	4.893735162	1.601599577	40.53228917	0.401311913	10.15375131	17.13620468	4.749054944
LT_DL 1 II	922	Darscholacke	Lotus.tenuis	DL	LT.DL922	8.1	8.75	0.151604	2019	2.126416496	1.073964811	11.85582956	0.304473206	2.227450614	4.520702569	3.646104813
LT_DL2 I	922	Darscholacke	Lotus.tenuis	DL	LT.DL922	8.4	8.63	0.078716	519.2	0.661436645	0.450111774	2.214132267	0.156862228	0.16398474	0.441537748	1.77351555
LT_DL3 II	922	Darscholacke	Lotus.tenuis	DL	LT.DL922	8.31	8.64	0.092798	1210	1.326593332	0.750186458	6.201839445	0.183750939	1.071156382	2.470774939	2.263221852
LT_SML1 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT.SML422	7.7	8.98	0.069312	1451	0.308813	0.335576477	12.21159223	0.259280077	1.391290398	0.856787857	5.327392575
LT_SML2 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT.SML422	7.74	8.76	0.067463	1372	0.253054267	0.258564282	11.37128569	0.185267729	1.874390871	0.87997386	4.472725964
LT_SML 3 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT.SML422	7.67	8.81	0.076526	1628	0.327854392	0.355246171	14.08757397	0.200672597	2.212202193	0.882832856	5.838289894
LT_SML1 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT.SML922	8.27	8.82	0.092349	2288	0.376284672	0.250881674	19.37124117	0.13693669	2.877156764	2.920461132	6.022215761
LT_SML2 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT.SML922	7.96	8.7	0.223802	1544	0.191924258	0.220778002	13.59904213	0.06953705	1.629628354	1.445068372	4.987109303
LT_SML3 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT.SML922	8.31	8.95	0.142578	2142	0.177147827	0.171182986	19.37834228	0.025887005	3.968703787	2.282460383	5.783633176

Table S25 Metabolites concentration of *Lotus tenuis* from Neusiedler See-Seewinkel

Concentrations are in µg/mg of dried weight (pigments, phenols, flavonoids, proteins); nmol/mg of dried weight (soluble sugars) and µmol/mg of dried weight (free amino acids, proline). Deleted outliers; replicate : April: LT_AM7, LT_DL10; September : LT_SV7 II , LT_DL9 II, LT_DL8 II , LT_SML 9). *ChlA = chlorophyll a, ChlB = chlorophyll b, Carot = carotenoids, Phe = phenols, Flav = flavonoids, SS = soluble sugars, AA = free amino acids, Pro = proline, Prot = proteins*

name	Season	Site	Species	ST	SP	Sample.group	ChlA	ChlB	Carot	Phe	Flav	SS	AA	Pro	Prot
LT_AM1	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	25.33024	7.341084	6.133079	24.70855	61.06027	323.1812	41.39748	151.4905	2.759046
LT_AM10	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	18.34855	6.344143	5.185561	21.83368	52.45302	239.0439	52.19588	223.7572	4.821784
LT_AM2	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	19.14832	5.974552	4.874538	24.81233	52.42171	401.2113	60.30937	271.9061	3.841205
LT_AM3	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	29.87445	10.57276	7.59563	28.7869	62.00343	405.9404	85.99821	457.8382	4.877073
LT_AM4	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	22.24901	7.640181	6.048705	25.89294	43.93785	337.037	71.07226	359.2982	2.482085
LT_AM5	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	17.55312	5.903907	3.87686	23.54596	49.41133	271.3932	40.60476	205.0371	2.888752
LT_AM6	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	18.20355	5.491868	4.191871	23.11378	53.02983	443.2832	102.2374	593.7809	4.650482
LT_AM8	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	13.81721	5.326585	3.45578	27.101	32.23607	255.8793	66.27756	407.7211	5.130601
LT_AM9	422	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM422	17.87136	6.40263	5.056802	21.98604	51.79956	267.2827	69.21258	341.5224	3.363907
LT_SV1	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	31.0393	8.666106	7.663601	24.24044	62.01706	377.748	114.8633	464.4448	1.49846
LT_SV10	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	37.33714	11.10164	10.14514	27.3259	70.39234	425.6371	85.56539	387.1955	6.319615
LT_SV2	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	38.5377	8.222925	9.365085	29.69317	61.70354	461.744	145.761	630.9156	2.34451
LT_SV3	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	56.31178	20.83105	6.030942	29.86869	64.80715	395.0842	96.61094	385.5877	3.083798
LT_SV4	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	58.59708	20.68099	6.732483	25.29038	56.09432	384.9408	145.6989	646.0544	5.433735
LT_SV5	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	48.10205	14.50998	9.48884	26.49207	67.65103	358.0041	115.5755	518.8835	3.036629
LT_SV6	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	47.80645	13.00652	11.56106	29.89672	70.34772	433.8964	160.4003	695.5262	5.990123
LT_SV7	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	33.45728	9.211607	7.479154	24.84171	61.14193	388.9225	126.2896	571.3553	2.24423
LT_SV8	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	40.37216	10.56825	10.09371	25.65472	70.81463	389.3241	105.9033	382.8245	4.584813
LT_SV9	422	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV422	32.20953	7.907591	8.476067	26.2541	56.23015	570.5017	136.9029	610.9334	4.279988
LT_DL1	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	20.76939	4.9483	5.025783	28.85716	82.68039	368.0546	38.8664	138.1653	0.363593
LT_DL2	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	28.84936	7.458068	7.858452	26.25355	151.8218	554.1934	78.94425	372.7672	0.508814
LT_DL3	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	32.21353	9.174519	8.660788	23.39684	90.1161	328.6991	77.36376	319.8664	2.142036
LT_DL4	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	24.855	5.937733	5.910777	22.7723	83.54458	261.5484	53.19752	239.6052	2.802839
LT_DL5	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	21.51321	4.571201	6.056092	23.80241	103.56	278.5411	32.73952	14.00728	1.337581
LT_DL6	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	19.31199	4.406559	5.084941	28.9509	103.775	290.5844	44.17069	172.1141	2.42447
LT_DL7	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	21.94424	5.531386	4.857938	16.79597	47.05371	262.7721	27.55949	45.85353	2.271096
LT_DL8	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	23.69406	6.259315	6.107088	25.60042	86.40432	441.6681	47.38559	146.4188	1.422555
LT_DL9	422	Darscholacke	Lotus.tenuis	DL	LT	LT.DL422	18.07127	3.835606	4.333701	29.05039	99.74313	244.0994	74.81634	360.8547	0.392729
LT_SML10	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	45.06441	11.38289	11.43476	25.86303	86.27605	543.016	137.7952	729.0133	4.455044
LT_SML1 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	27.79578	7.44973	8.553229	22.37796	78.23005	302.8906	90.02325	648.1798	3.58276
LT_SML2 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	31.55536	7.601419	9.16218	26.80297	58.98359	327.2793	98.82256	663.6867	4.024857
LT_SML 3 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	33.18648	8.052928	9.080362	21.34491	55.5225	351.4731	94.6228	623.3118	4.956257
LT_SML4 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	34.0142	8.474584	10.17503	29.04288	79.3104	454.5129	89.03296	584.5968	5.404413
LT_SML5 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	40.73367	9.472865	11.4884	20.94113	63.98107	490.7916	90.72316	609.7345	3.716014
LT_SML 6 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	32.69101	6.663192	9.278392	22.6017	39.83569	513.7671	116.4903	773.1986	5.072845
LT_SML7 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	53.14089	10.4611	10.83881	27.53205	67.90299	384.6647	100.3597	591.4629	2.153167
LT_SML8 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	35.91861	9.399273	10.02114	29.11416	68.61222	326.1178	125.6868	750.086	5.967432
LT_SML9 I	422	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML422	46.09669	11.8704	12.65346	31.1867	73.69229	408.5256	154.1253	847.5472	2.457264
LT_AM10 I	922	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM922	36.15144	9.093633	10.60899	30.44853	75.99156	277.9895	125.3406	279.0863	8.349557
LT_AM2 I	922	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM922	32.73796	7.908387	9.509066	24.43439	68.63116	282.7104	59.01297	374.5681	3.587679
LT_AM3 II	922	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM922	38.85607	10.19016	10.86744	19.77895	59.4788	267.4995	51.16979	241.9247	4.030999
LT_AM 4 I	922	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM922	32.35225	8.523734	9.607554	28.85234	66.8645	328.5773	52.35417	273.3381	9.472058
LT_AM 5 I	922	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM922	27.94853	8.475856	8.783485	27.45696	75.9681	343.7125	49.9058	325.4341	6.313379
LT_AM7 II	922	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM922	32.10768	8.72843	9.237147	26.57471	79.8294	322.6947	81.00745	88.10753	7.07851
LT_AM9 I	922	AlteMuehle	Lotus.tenuis	AM	LT	LT.AM922	39.70084	12.30987	10.60178	26.12482	60.20661	363.347	132.5104	138.0001	15.00052
LT_SV1 I	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	40.2751	10.73695	11.34515	24.71511	66.13614	278.3225	89.9226	512.9388	7.213163
LT_SV10 II	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	40.89308	10.76392	10.95988	27.10019	75.50781	426.6733	160.8614	370.3183	7.220522
LT_SV2 I	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	45.82911	9.895224	13.35352	31.65433	94.25358	288.9551	82.27884	440.9975	5.773337
LT_SV3 II	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	44.92867	10.93671	12.79434	24.63695	69.97056	299.6704	46.50898	263.8102	8.249665
LT_SV4 III	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	43.07265	10.94959	12.23407	24.36061	70.34783	394.5711	50.25205	230.4935	5.879188
LT_SV5 I	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	38.48164	11.1729	10.29715	24.53491	62.94011	313.338	38.77128	151.1996	3.641575
LT_SV6 II	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	46.43608	12.55166	12.84972	25.98197	72.57573	318.3978	90.0224	683.8245	11.58005
LT_SV8 I	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	42.74426	10.51842	12.45352	25.8783	68.35547	273.3124	106.0098	812.4643	9.965587
LT_SV9 I	922	Seevorgelaende	Lotus.tenuis	SV	LT	LT.SV922	46.36792	11.73222	12.77828	25.7081	73.69706	281.4548	75.50745	561.4665	8.168416
LT_DL 1 II	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	41.068	10.61979	11.35747	30.82602	96.24578	281.8064	55.41227	346.6786	11.44543
LT_DL10 I	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	32.79455	10.1949	10.40151	25.95846	78.85394	237.8275	55.41476	309.9839	8.955591
LT_DL2 I	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	47.60002	11.43531	13.60958	24.61544	84.00005	289.7386	78.50105	448.6871	7.222036
LT_DL3 II	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	30.68288	8.895755	9.789417	29.22912	80.57736	282.0046	84.76137	667.009	11.69534
LT_DL4 II	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	37.90422	10.81028	11.10519	29.62459	85.86786	301.6778	94.82715	118.3344	7.133171
LT_DL5 I	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	50.57558	13.71748	13.91778	25.40507	104.5289	261.4454	66.23941	337.6642	6.764989
LT_DL6 I	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	34.19915	9.344849	9.844758	31.72607	90.51225	299.0087	60.68171	408.5194	9.394398
LT_DL7 I	922	Darscholacke	Lotus.tenuis	DL	LT	LT.DL922	42.20173	10.18595	12.97938	27.12465	77.02873	337.8589	61.00282	299.5208	8.85157
LT_SML1 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	47.2892	12.58118	12.722	22.80566	68.27594	286.8756	50.77184	200.841	9.840655
LT_SML2 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	43.16323	9.90621	12.42528	25.51089	67.21995	289.3846	59.72526	332.515	8.152025
LT_SML 3 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	29.96264	8.569326	8.840702	28.45875	78.89173	244.4771	56.49733	361.4914	8.426341
LT_SML4 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	44.48834	10.24713	13.32499	25.15136	70.10935	223.9689	49.10131	244.8605	7.953804
LT_SML5 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	47.53521	10.16821	13.65773	28.99109	80.09761	348.0751	61.4707	300.0112	7.458759
LT_SML 6 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	30.61474	8.824911	8.685729	25.13273	60.88999	300.1729	84.05351	84.9383	7.093123
LT_SML 7 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	41.77679	10.30049	12.36937	25.79426	71.32688	254.7884	51.39895	339.5228	9.409285
LT_SML8 I	922	Sechsmahdlacke	Lotus.tenuis	SML	LT	LT.SML922	34.41924	9.749638	9.747391	23.71061	56.59219	260.9025	83.8031	76.44106	9.280263

Table S26 Metabolite concentration of *Lotus tenuis* and *Lotus japonicus* controls

Concentrations are in µg/mg of dried weight (pigments, phenols, flavonoids, proteins); nmol/mg of dried weight (soluble sugars) and µmol/mg of dried weight (free amino acids, proline). Deleted outliers; replicate LJ7_Nfed ; LJ1_Nfed. *ChlA = chlorophyll a, ChlB = chlorophyll b, Carot = carotenoids, Phe = phenols, Flav = flavonoids, SS = soluble sugars, AA = free amino acids, Pro = proline, Prot = proteins*

name	Season	Site	Species	ST	Sample.group	ChlA	ChlB	Carot	Phe	Flav	AA	Pro	SS	Prot
LJ2_Nfed	LJ.Nfed.G(	LJ.Nfed.G(	Lotus.japonicus	Nfed	LJ.Nfed	19.98815	5.647286	5.172403	15.44442	25.87384	84.13362	440.4122	158.9498	4.471547
LJ3_Nfed	LJ.Nfed.G(	LJ.Nfed.G(	Lotus.japonicus	Nfed	LJ.Nfed	27.5787	8.314985	6.069064	15.90404	31.83885	101.2121	480.2234	235.0769	4.034146
LJ4_Nfed	LJ.Nfed.G(	LJ.Nfed.G(	Lotus.japonicus	Nfed	LJ.Nfed	28.66886	7.365394	7.757588	14.28031	32.75517	46.61514	162.5467	189.7298	3.012447
LJ5_Nfed	LJ.Nfed.G(	LJ.Nfed.G(	Lotus.japonicus	Nfed	LJ.Nfed	29.17505	7.290185	7.581562	14.41978	31.95953	38.43791	206.7055	200.6201	2.964326
LJ6_Nfed	LJ.Nfed.G(	LJ.Nfed.G(	Lotus.japonicus	Nfed	LJ.Nfed	31.32961	7.455989	8.063192	15.02743	32.06185	84.08176	488.8187	213.372	5.622172
LJ8_Nfed	LJ.Nfed.G(	LJ.Nfed.G(	Lotus.japonicus	Nfed	LJ.Nfed	23.67339	6.395465	6.028047	15.24526	27.82949	91.43436	481.7729	229.6866	4.274003
LT1_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	35.8181	9.57661	9.717537	18.4119	55.17145	47.5169	250.533	145.9972	3.099301
LT2_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	22.17163	5.881194	6.226591	11.86548	26.49087	24.85409	154.512	138.4462	3.689933
LT3_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	35.36583	8.767045	9.076973	17.54684	40.81283	37.23082	129.9412	166.581	4.344924
LT4_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	22.13702	6.301203	6.427207	14.66508	40.05479	89.34102	567.119	183.0905	3.565559
LT5_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	30.27028	7.91656	8.342895	17.1	30.25794	57.96145	346.8339	158.0281	3.571724
LT6_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	33.58014	8.551975	9.030478	14.56872	38.78374	67.48029	393.6719	217.3158	5.970482
LT7_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	24.08347	6.664864	6.706624	14.81453	34.8495	40.37047	205.6477	154.3776	3.604517
LT8_Nfed	LT.Nfed.G(	LT.Nfed.G(	Lotus.tenuis.c	Nfed	LT.Nfed	25.90678	6.665363	7.01031	13.51832	27.45113	42.23131	261.6948	145.8819	3.066176
LJ1_Nfix	LJ.Nfix.GC	LJ.Nfix.GC	Lotus.japonicus	Nfix	LJ.Nfix	22.66507	7.067083	5.770446	15.69518	38.48548	48.61188	235.2958	205.6924	4.143992
LJ2_Nfix	LJ.Nfix.GC	LJ.Nfix.GC	Lotus.japonicus	Nfix	LJ.Nfix	18.17621	5.209382	4.813952	15.16453	35.49172	25.85174	132.1363	163.4266	4.337316
LJ3_Nfix	LJ.Nfix.GC	LJ.Nfix.GC	Lotus.japonicus	Nfix	LJ.Nfix	17.62593	5.030314	4.113364	15.97577	31.59711	42.78169	211.3493	185.5134	2.966148
LJ4_Nfix	LJ.Nfix.GC	LJ.Nfix.GC	Lotus.japonicus	Nfix	LJ.Nfix	15.04603	4.661096	3.598255	14.68158	31.3723	38.96537	177.5328	198.0216	4.243588
LJ5_Nfix	LJ.Nfix.GC	LJ.Nfix.GC	Lotus.japonicus	Nfix	LJ.Nfix	13.77117	4.623823	3.311513	19.18882	52.70021	48.92358	226.3798	192.4065	1.072884
LJ7_Nfix	LJ.Nfix.GC	LJ.Nfix.GC	Lotus.japonicus	Nfix	LJ.Nfix	13.86289	6.592052	2.637285	17.98429	36.4727	56.20802	325.4644	226.8389	3.073287
LJ8_Nfix	LJ.Nfix.GC	LJ.Nfix.GC	Lotus.japonicus	Nfix	LJ.Nfix	22.35492	6.64698	5.122676	15.31293	35.92879	42.59718	257.5419	211.0062	1.280676
LT1_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	26.95549	7.116183	7.324696	20.16456	52.73244	110.9934	625.2264	220.7851	2.002847
LT2_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	51.54755	12.33969	12.562	15.52859	42.6726	94.19646	447.2931	199.5291	1.589387
LT3_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	49.4601	14.52128	11.99781	21.52277	56.23302	109.1439	628.3615	246.0494	8.216959
LT4_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	41.10545	11.9056	10.59661	21.31306	56.31765	76.82002	424.8212	191.2537	5.730026
LT5_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	45.03385	12.77356	11.52712	23.8416	59.23313	115.5749	671.2138	172.9024	6.537621
LT6_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	27.88126	8.181823	6.794874	19.07635	42.99454	84.49248	527.8972	217.9394	5.769514
LT7_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	29.2261	8.984924	7.554559	22.8345	51.75561	101.3714	575.6931	336.1124	3.795012
LT8_Nfix	LT.Nfix.GC	LT.Nfix.GC	Lotus.tenuis.c	Nfix	LT.Nfix	29.02989	9.227747	6.601666	16.68606	44.64436	51.32043	348.1623	215.2819	5.699512

Table S27 Metabolites data used for RDA analysis, *Lepidium cartilagineum*

Metabolites of first 3 replicates – concentrations, used only for RDA analysis. Concentrations are in µg/mg of dried weight (pigments, phenols, flavonoids, proteins); nmol/mg of dried weight (soluble sugars) and µmol/mg of dried weight (free amino acids, proline). *ChlA = chlorophyll a, ChlB = chlorophyll b, Carot = carotenoids, Phe = phenols, Flav = flavonoids, SS = soluble sugars, AA = free amino acids, Pro = proline, Prot = proteins*

name	Season	Site	Species	ST	Sample.group	ChlA	ChlB	Carot	Phe	Flav	AA	Pro	SS	Prot
LC_AM1	422	AlteMuehle	Lepidium.cartilagineum	AM	LC.AM422	19.24691	5.354127	5.768739	33.56689	59.71618	634.0636	4008.039	332.5354	1.762916
LC_AM2	422	AlteMuehle	Lepidium.cartilagineum	AM	LC.AM422	22.79899	6.145358	6.750396	30.12541	69.4546	535.9168	3441.573	433.2574	3.00189
LC_AM3	422	AlteMuehle	Lepidium.cartilagineum	AM	LC.AM422	17.75378	5.968793	4.962721	29.12248	66.21882	486.3544	3169.806	412.6478	1.408537
LC_AM1 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC.AM922	34.77327	8.797358	11.14675	39.72812	92.35155	543.9949	3207.846	285.6092	31.6749
LC_AM2 III	922	AlteMuehle	Lepidium.cartilagineum	AM	LC.AM922	34.02994	10.76079	10.6474	36.26424	72.44058	630.9946	3587.87	384.0518	34.48367
LC_AM3 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC.AM922	30.52715	9.347048	9.480583	36.50128	73.25469	475.3721	2814.543	312.4277	16.62305
LC_SV1	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC.SV422	40.01201	9.702475	10.67048	33.52226	83.65113	718.2005	4606.688	277.3092	2.899605
LC_SV2	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC.SV422	31.52269	7.594209	8.358686	36.38022	92.57295	388.5563	2533.103	292.9491	3.305267
LC_SV3	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC.SV422	33.95675	8.045557	11.16846	32.29049	107.1911	403.4511	2634.625	403.3712	1.417034
LC_SV1 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC.SV922	44.78165	11.51248	13.11525	37.92649	80.31863	443.8041	2574.219	203.6705	28.25135
LC_SV2 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC.SV922	36.42675	13.48017	11.78964	53.53685	107.413	698.7551	4155.653	277.1911	54.82168
LC_SV3 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC.SV922	21.91383	8.48507	8.52044	31.49277	59.83771	338.3477	1954.749	202.1064	9.941257

Table S28 Soil data used for analysis RDA, Tukey HSD multiple comparison, *Lepidium cartilagineum*

Concentrations are given in decimal percentages for soil water content; Ece (mS/cm) for soil salinity; pH for water and $CaCl_2$ and mM/L for all soil ions (Mg^{2+} , Ca^{2+}, Na^{2+}, K^{+}, Cl^{-}, SO_4^{2-}, CO_3^{2-});
SWC_BD = soil water content

name	Season	Site	Species	ST	Sample.gr	pH_CaCl2	pH_H2O	SWC_BD	conductivity	magnesium_mM	calcium_mM	sodium_mM	potassium_mM	chloride_mM	sulfate_mM	carbonate_mM
LC_AM1	422	AlteMueh	Lepidium.	AM	LC.AM422	8.44	9.2	0.132446	13750	0.061527401	0.05162031	142.0509734	0.981109072	24.55912576	31.37000453	27.97962153
LC_AM2	422	AlteMueh	Lepidium.	AM	LC.AM422	8.23	9.23	0.140621	13750	0.043952138	0.042287266	156.1960135	0.998571727	28.07013734	28.12103179	36.52743157
LC_AM3	422	AlteMueh	Lepidium.	AM	LC.AM422	8.49	9.22	0.154218	11210	0.066052553	0.062568753	120.7527441	0.856646739	24.10933975	24.22739247	24.6512544
LC_AM1 II	922	AlteMueh	Lepidium.	AM	LC.AM922	9.13	9.16	0.151614	11610	0.070480276	0.077399226	119.2055533	0.778952734	23.77190493	22.52693596	25.72724411
LC_AM2 II	922	AlteMueh	Lepidium.	AM	LC.AM922	8.35	8.606	0.186056	7306	0.147137694	0.164811232	65.91249169	0.690377037	16.28468637	12.93437752	12.53666258
LC_AM3 II	922	AlteMueh	Lepidium.	AM	LC.AM922	8.84	9.12	0.132473	12410	0.084868994	0.080565511	118.091691	1.043195749	25.27919803	26.36124149	20.73203738
LC_SV1	422	Seevorgel	Lepidium.	SV	LC.SV422	8.18	9.56	0.131269	15920	0.045496201	0.022931568	167.2376998	2.327592404	14.45430825	16.75634822	60.86757151
LC_SV2	422	Seevorgel	Lepidium.	SV	LC.SV422	8.16	9.62	0.19887	11880	0.030133703	0.021822026	121.3946517	2.007929435	13.69813905	11.65148481	43.25269198
LC_SV3	422	Seevorgel	Lepidium.	SV	LC.SV422	8.03	9.47	0.138292	13820	0.029694591	0.019167453	143.3689325	1.927491249	15.68266332	12.3911935	52.46454875
LC_SV1 I	922	Seevorgel	Lepidium.	SV	LC.SV922	9.21	9.9	0.078037	19450	0.038340981	0.020594192	215.0941249	2.425817003	20.9433222	25.18413437	73.16311066
LC_SV2 I	922	Seevorgel	Lepidium.	SV	LC.SV922	8.88	9.58	0.184589	15670	0.046968313	0.036676569	175.7115353	2.007173302	14.38754457	17.02287422	64.72635266
LC_SV3 I	922	Seevorgel	Lepidium.	SV	LC.SV922	8.43	9.52	0.22904	11410	0.03392453	0.029942097	116.9845461	1.625736397	13.86802681	12.13016422	40.30483027

Table S29 Metabolites concentration of *Lepidium cartilagineum* from Neusiedler See-Seewinkel

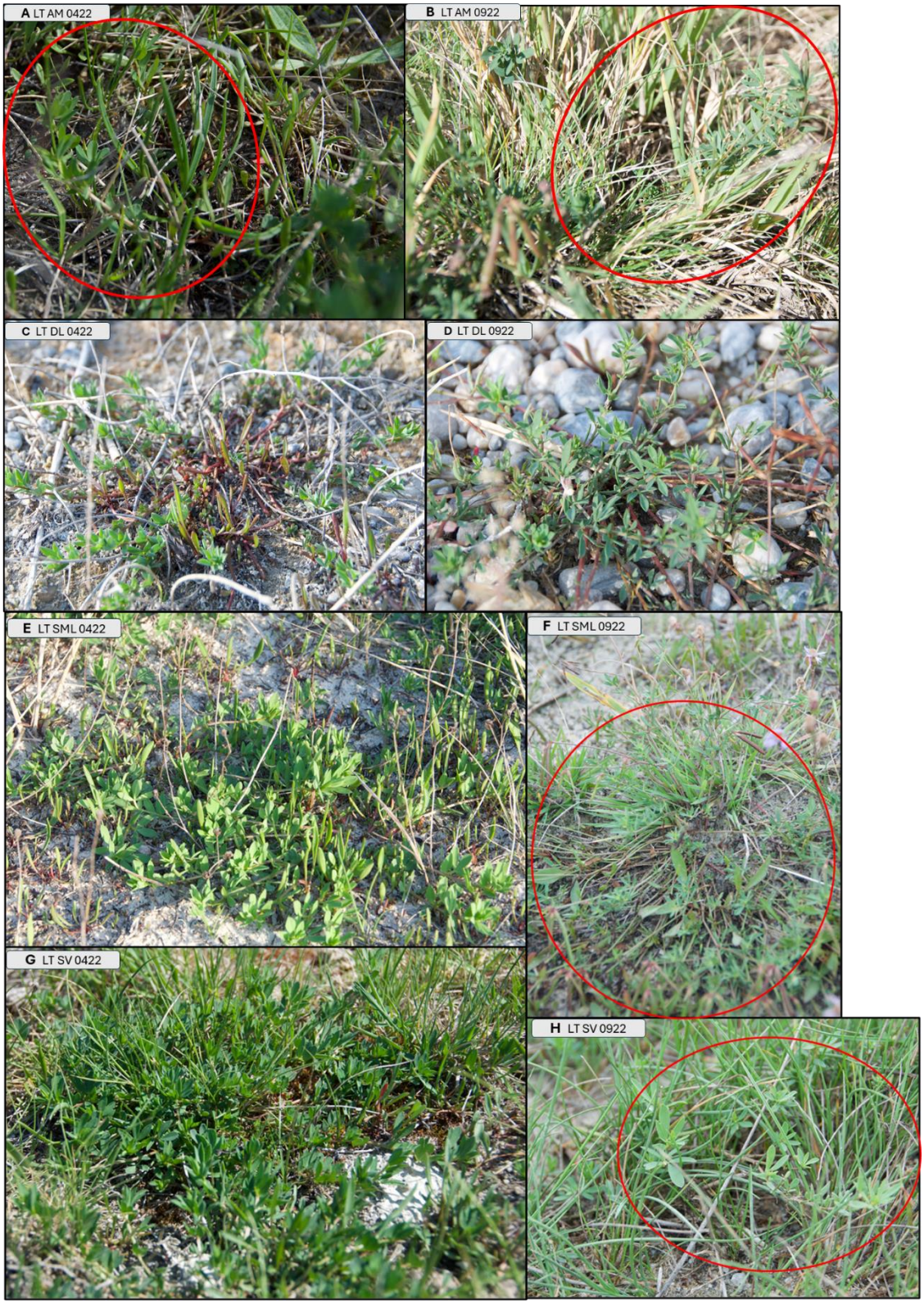
Concentrations are in µg/mg of dried weight (pigments, phenols, flavonoids, proteins); nmol/mg of dried weight (soluble sugars) and µmol/mg of dried weight (free amino acids, proline).
ChlA = chlorophyll a, ChlB = chlorophyll b, Carot = carotenoids, Phe = phenols, Flav = flavonoids, SS = soluble sugars, AA = free amino acids, Pro = proline, Prot = proteins

name	Season	Site	Species	ST	SP	Sample.group	ChlA	ChlB	Carot	Phe	Flav	SS	AA	Pro	Prot
LC_AM1	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	19.24691	5.354127	5.768739	33.56689	59.71618	332.5354	634.0636	4008.039	1.762916
LC_AM10	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	20.936	6.084055	6.373264	25.24	63.31326	304.332	803.5907	5115.58	2.41484
LC_AM2	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	22.79899	6.145358	6.750396	30.12541	69.4546	433.2574	535.9168	3441.573	3.00189
LC_AM3	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	17.75378	5.968793	4.962721	29.12248	66.21882	412.6478	486.3544	3169.806	1.408537
LC_AM4	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	20.59601	6.976644	5.736528	28.91048	88.18885	464.0179	286.3842	1838.4	2.314165
LC_AM5	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	21.6328	6.2006	6.242408	32.56545	78.1469	384.268	625.7932	4032.118	4.637161
LC_AM6	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	15.90014	4.135344	4.269109	30.82519	77.71202	357.2942	460.5069	2905.174	3.520972
LC_AM7	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	17.0205	4.098718	4.896901	31.6201	84.99339	363.3267	362.61	2329.815	1.13867
LC_AM8	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	17.16773	4.783734	5.164225	29.36977	70.0533	419.4621	254.1049	1605.398	1.089245
LC_AM9	422	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM422	18.47777	4.958016	4.877875	35.40921	63.69351	316.774	966.1405	6147.569	1.894723
LC_SV1	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	40.01201	9.702475	10.67048	33.52226	83.65113	277.3092	718.2005	4606.688	2.899605
LC_SV10	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	34.62649	8.48039	10.36389	33.18369	90.17623	409.3403	568.6661	3792.484	1.683462
LC_SV2	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	31.52269	7.594209	8.358686	36.38022	92.57295	292.9491	388.5563	2533.103	3.305267
LC_SV3	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	33.95675	8.045557	11.16846	32.29049	107.1911	403.3712	403.4511	2634.625	1.417034
LC_SV4	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	31.99384	7.580495	9.624205	38.21118	95.82614	348.7895	432.4467	2823.551	1.876151
LC_SV5	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	19.48289	5.528521	6.291593	32.17668	83.00332	455.8275	337.7068	2194.116	1.561471
LC_SV6	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	35.37996	7.856679	9.750465	40.06657	93.63582	334.7982	492.7566	3215.547	5.726743
LC_SV7	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	26.77469	6.619319	7.902812	35.99785	83.47722	429.4292	311.9022	2067.891	2.911768
LC_SV8	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	29.89804	7.274805	8.503464	34.01091	80.89598	376.6623	458.0836	2977.159	4.391291
LC_SV9	422	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV422	33.5327	7.933857	9.992894	35.87502	91.21307	361.4903	584.9808	3876.099	2.319361
LC_AM1 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	34.77327	8.797358	11.14675	39.72812	92.35155	285.6092	543.9949	3207.846	31.6749
LC_AM 10	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	29.46166	8.689817	9.5207	34.43553	69.76899	272.9603	536.3952	2862.779	2.387373
LC_AM2 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	34.02994	10.76079	10.6474	36.26424	72.44058	384.0518	630.9946	3587.87	34.48367
LC_AM3 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	30.52715	9.347048	9.480583	36.50128	73.25469	312.4277	475.3721	2814.543	16.62305
LC_AM4 I	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	37.66502	8.515662	11.81788	38.9381	70.95961	267.3523	617.2242	3652.882	13.59555
LC_AM5 I	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	35.30476	10.0885	10.54912	38.67012	77.58326	320.1932	614.7253	3656.956	42.58096
LC_AM6 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	28.13143	7.467368	9.578118	40.09681	75.04063	320.2738	710.0078	3757.128	6.796104
LC_AM7 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	28.53529	8.21203	9.004077	39.50879	72.11446	299.0344	621.7539	3230.717	10.97292
LC_AM8 I	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	33.36952	9.849336	10.10047	36.51726	74.74211	354.3397	339.5214	1975.861	4.282919
LC_AM9 II	922	AlteMuehle	Lepidium.cartilagineum	AM	LC	LC.AM922	24.60529	7.135201	7.841815	39.27914	69.34168	290.8434	480.4056	2384.292	0.605745
LC_SV1 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	44.78165	11.51248	13.11525	37.92649	80.31863	203.6705	443.8041	2574.219	28.25135
LC_SV10 II	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	40.30748	8.938306	11.9269	44.84899	87.47317	252.2324	686.2046	3630.138	4.084222
LC_SV2 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	36.42675	13.48017	11.78964	53.53685	107.413	277.1911	698.7551	4155.653	54.82168
LC_SV3 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	21.91383	8.48507	8.52044	31.49277	59.83771	202.1064	338.3477	1954.749	9.941257
LC_SV4 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	32.07663	9.749019	11.56488	40.375	80.56515	232.5946	544.3964	3057.327	19.44564
LC_SV5 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	33.14457	8.616459	10.37436	37.55179	73.24186	196.3	330.1722	1815.025	12.00204
LC_SV6 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	26.61929	7.293763	8.694108	40.54259	69.99071	258.5448	351.2992	1580.675	10.95257
LC_SV7 II	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	36.14413	9.748553	12.32443	41.59903	87.64075	241.8055	612.1499	3399.949	11.18572
LC_SV8 I	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	42.68669	10.02731	12.9109	39.45157	78.92859	262.4624	596.5104	3031.162	14.49706
LC_SV9 II	922	Seevorgelaende	Lepidium.cartilagineum	SV	LC	LC.SV922	42.19618	12.21779	12.72198	46.41046	86.42363	259.5369	557.505	2911.557	1.084689

Table S30 Metabolite concentration of *Lepidium cartilagineum* and *Lepidium sativum* controls Concentrations are in µg/mg of dried weight (pigments, phenols, flavonoids, proteins); nmol/mg of dried weight (soluble sugars) and µmol/mg of dried weight (free amino acids, proline). Deleted outliers; LS_GC2. *ChlA = chlorophyll a, ChlB = chlorophyll b, Carot = carotenoids, Phe = phenols, Flav = flavonoids, SS = soluble sugars, AA = free amino acids, Pro = proline, Prot = proteins*

name	Season	Site	Species	ST	Sample.group	ChlA	ChlB	Carot	Phe	Flav	AA	Pro	SS	Prot
LC_GC1	LC.GC	LC.GC	Lepidium.cartilagineum.c	GC	LC.GC	48.64139	11.91454	11.28538	21.87271	62.21249	505.9912	2826.744	139.0533	10.72651
LC_GC3	LC.GC	LC.GC	Lepidium.cartilagineum.c	GC	LC.GC	48.82236	12.77494	11.13968	27.05366	74.65688	521.2771	3027.883	189.5112	13.04596
LC_GC4	LC.GC	LC.GC	Lepidium.cartilagineum.c	GC	LC.GC	53.268	14.29661	11.15714	19.04226	59.16395	575.1558	3337.933	218.0818	10.41275
LC_GC5	LC.GC	LC.GC	Lepidium.cartilagineum.c	GC	LC.GC	38.64014	9.448506	9.872781	24.15146	66.20633	456.7877	2672.88	148.3749	11.33117
LC_GC6	LC.GC	LC.GC	Lepidium.cartilagineum.c	GC	LC.GC	60.4821	14.41781	12.98395	21.6962	57.63881	652.9812	3887.799	171.7675	4.133432
LC_GC7	LC.GC	LC.GC	Lepidium.cartilagineum.c	GC	LC.GC	53.3822	12.8336	12.22957	29.05679	68.4969	575.7246	3451.02	223.6381	4.696932
LC_GC8	LC.GC	LC.GC	Lepidium.cartilagineum.c	GC	LC.GC	50.37694	12.71797	11.16516	20.78325	59.46104	524.5431	3108.734	180.1262	6.094868
LS_GC1	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	29.44193	7.919113	6.22304	12.32489	73.59883	57.84993	224.6244	139.0126	10.08536
LS_GC2	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	48.90869	12.19567	9.543623	13.20996	78.1814	92.87258	369.7673	160.1939	15.42454
LS_GC3	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	17.45358	4.950382	4.790872	12.14474	68.71301	28.59365	133.659	129.4076	7.645839
LS_GC4	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	14.52479	4.803728	3.413536	11.55061	58.71149	39.15995	193.6462	152.8166	2.474956
LS_GC5	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	20.53663	6.146744	5.517214	9.558363	55.22058	32.46569	153.1769	124.13	1.137489
LS_GC6	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	20.03179	6.040734	4.141707	15.96332	87.20181	50.80205	253.0705	166.7405	1.883776
LS_GC7	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	15.30652	4.470377	3.533459	14.17932	72.50949	45.02191	249.9467	163.8413	3.911103
LS_GC8	LS.GC	LS.GC	Lepidium.sativum	GC	LS.GC	22.34279	6.571392	5.835961	10.61143	54.84115	53.6493	315.6062	222.1538	2.260824

Pictures of analysed plants species and controls



Picture S1 *Lotus tenuis* wild samples from Seewinkel

In red circles are depicted LT plants to distinguish them on grasslands. *LT- Lotus tenuis, AM = Alte*

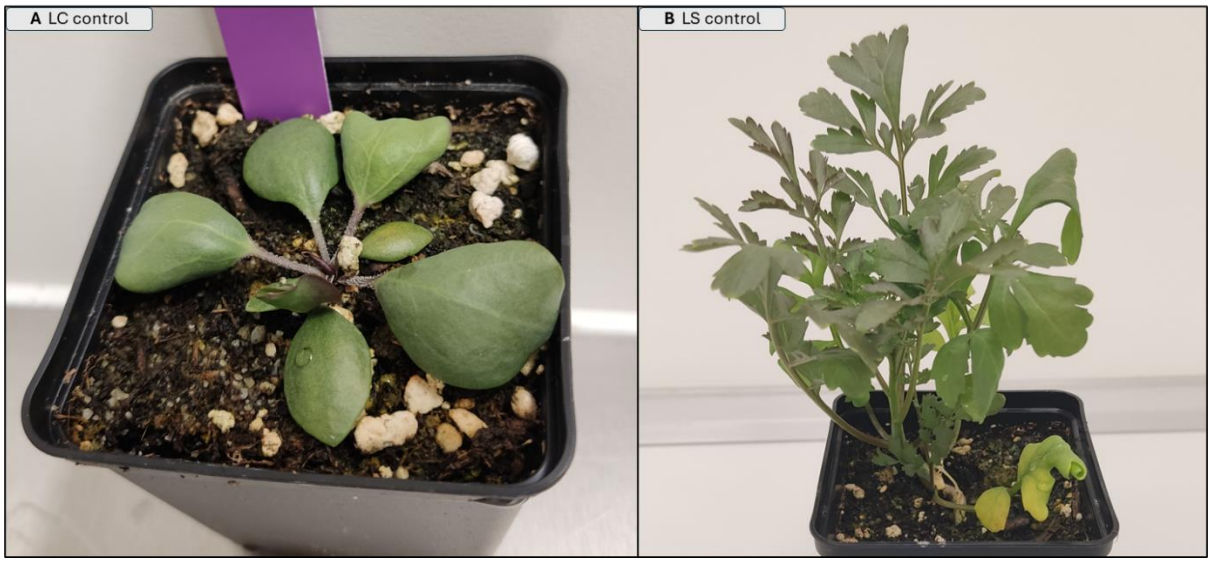
Mühle, DL = Darscholacke, SML = Sechsmahdlacke, SV = Seevorgelände, 0422 = April, 0922 = September.

Picture S2 *Lotus tenuis* and *Lotus japonicus* control plants across both regimes

Note that Lotus tenuis seeds were collected from Darscholacke site. LJ = Lotus japonicus, LT = Lotus tenuis, N-fix – inoculated nitrogen fixing plants, N-fed = nitrogen fed plants, with supressed nodulation.

Picture S3 *Lepidium cartilagineum* wild samples from Seewinkel

Note, that compared to LT, at AM and SV site, Lepidium grows alone, not surrounded by other plants. *LC = Lepidium cartilagineum, AM = Alte Mühle; SV = Seevorgelände, 0422 = April, 0922 = September.*



Picture S4 *Lepidium cartilagineum* and *Lepidium sativum* control plants

Note the differences in thickness of *Lepidium cartilagineum* leaves, compared to outside plants – controls are thinner, less meaty.

References

Abdel-Lateif, K., Bogusz, D., & Hocher, V. (2012). The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and Frankia bacteria. *Plant Signaling & Behavior*, *7*(6), 636–641. https://doi.org/10.4161/psb.20039

Adams, R. M., Hurd, B. H., Lenhart, S., & Leary, N. (1998). Effects of global climate change on agriculture: An interpretative review. *Clim Res*.

Al Hassan, M., Estrelles, E., Soriano, P., López-Gresa, M. P., Bellés, J. M., Boscaiu, M., & Vicente, O. (2017). Unraveling Salt Tolerance Mechanisms in Halophytes: A Comparative Study on Four Mediterranean Limonium Species with Different Geographic Distribution Patterns. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.01438

Albert, R., & Kinzel, H. (1973). Unterscheidung von physiotypen bei halophyten des neusiedlerseegebietes (Österreich). *Zeitschrift für Pflanzenphysiologie*, *70*(2), 138–157. https://doi.org/10.1016/S0044-328X(73)80057-1

Albert, R., Werner, V., Popp, M., Fischer, M., & Niklfeld, H. (2020). *Botanische Kostbarkeiten vor unserer Haustür Die Salzpflanzen im Gebiet des Neusiedler Sees, Burgenland (Österreich)*. *157*, 115–143.

Amine-Khodja, I. R., Boscari, A., Riah, N., Kechid, M., Maougal, R. T., Belbekri, N., & Djekoun, A. (2022). Impact of Two Strains of Rhizobium leguminosarum on the Adaptation to Terminal Water Deficit of Two Cultivars Vicia faba. *Plants*, *11*(4), Article 4. https://doi.org/10.3390/plants11040515

Araújo, W. L., Tohge, T., Ishizaki, K., Leaver, C. J., & Fernie, A. R. (2011). Protein degradation – an alternative respiratory substrate for stressed plants. *Trends in Plant Science*, *16*(9), 489–498. https://doi.org/10.1016/j.tplants.2011.05.008

Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59*(2), 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Atilio J., B., & Causin, H. F. (1996). The central role of amino acids on nitrogen utilization and plant growth. *Journal of Plant Physiology*, *149*(3), 358–362. https://doi.org/10.1016/S0176-1617(96)80134-9

Bacha, H., Tekaya, M., Drine, S., Guasmi, F., Touil, L., Enneb, H., Triki, T., Cheour, F., & Ferchichi, A. (2017). Impact of salt stress on morpho-physiological and biochemical parameters of Solanum lycopersicum cv. Microtom leaves. *South African Journal of Botany*, *108*, 364–369. https://doi.org/10.1016/j.sajb.2016.08.018

Barbafieri, M., Bretzel, F., Scartazza, A., Di Baccio, D., Rosellini, I., Grifoni, M., Pini, R., Clementi, A., & Franchi, E. (2023). Response to Hypersalinity of Four Halophytes Growing in Hydroponic Floating Systems: Prospects in the Phytomanagement of High Saline Wastewaters and Extreme Environments. *Plants*, *12*(9), 1737. https://doi.org/10.3390/plants12091737

Bates, L. S., Waldren, R. a, & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*, 205–207.

Bertrand, A., Bipfubusa, M., Dhont, C., Chalifour, F.-P., Drouin, P., & Beauchamp, C. J. (2016). Rhizobial strains exert a major effect on the amino acid composition of alfalfa nodules under NaCl stress. *Plant Physiology and Biochemistry*, *108*, 344–352. https://doi.org/10.1016/j.plaphy.2016.08.002

Bibi, N., Shah, M. H., Khan, N., Al-Hashimi, A., Elshikh, M. S., Iqbal, A., Ahmad, S., & Abbasi, A. M. (2022). Variations in Total Phenolic, Total Flavonoid Contents, and Free Radicals'

Scavenging Potential of Onion Varieties Planted under Diverse Environmental Conditions. *Plants*, *11*(7), 950. https://doi.org/10.3390/plants11070950

Black, C. A., Evans, D. D., White, J. L., Ensminger, L. E., & Clark, F. E. (1983). Methods of Soil Analysis. In A. L. Page (Ed.), *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*. American Society of Agronomy, Soil Science Society of America. https://doi.org/10.2134/agronmonogr9.2.2ed

Boros, E., Horváth, Zs., Wolfram, G., & Vörös, L. (2014). Salinity and ionic composition of the shallow astatic soda pans in the Carpathian Basin. *Annales de Limnologie - International Journal of Limnology*, *50*(1), 59–69. https://doi.org/10.1051/limn/2013068

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

Bueno, M., Lendínez, M. L., Calero, J., & del Pilar Cordovilla, M. (2020). Salinity responses of three halophytes from inland saltmarshes of Jaén (southern Spain). *Flora*, *266*, 151589. https://doi.org/10.1016/j.flora.2020.151589

Carillo, P., & Gibon, Y. (2011). Protocol: Extraction and determination of proline. *PrometheusWiki*, *2011*, 1–5.

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*(3).

Chaudhary, D. R. (2019). Ion accumulation pattern of halophytes. *Halophytes and Climate Change: Adaptive Mechanisms and Potential Uses*, 137–151. https://doi.org/10.1079/9781786394330.0137

Chutipaijit, S., Cha-Um, S., & Sompornpailin, K. (2011). High contents of proline and anthocyanin increase protective response to salinity in Oryza sativa L. spp. Indica. *Australian Journal of Crop Science*, *5*, 1191–1198.

Corwin, D. L. (2021). *Climate change impacts on soil salinity in agricultural areas*. *72*, 842–862. https://doi.org/10.1111/ejss.13010862 CORWIN

Dubey, R. S. (1999). Protein synthesis by plants under stressful conditions. In *Handbook of plant and crop stress* (2nd ed., pp. 365–397).

*eHALOPH | eHALOPH - Show Plant—*Lepidium cartilagineum . (n.d.). Retrieved 9 September 2024, from https://ehaloph.uc.pt/showplant/11954

*eHALOPH | eHALOPH - Show Plant—*Lotus tenuis. (n.d.). Retrieved 9 September 2024, from https://ehaloph.uc.pt/showplant/11410

Eitzinger, J., Kubu, G., Formayer, H., Haas, P., Gerersdorfer, T., & Kromp-Kolb, H. (2009). *Auswirkungen einer Klimaänderung auf den Wasserhaudhalt des Neusiedler Sees* (Endbericht Im Auftrag Der Burgenländischen Landesregierung Vom 15. Juli 2005 1; BOKU-Met Report). http://www.boku.ac.at/met/report/

Fitzner, M., Schreiner, M., & Baldermann, S. (2023). The interaction of salinity and light regime modulates photosynthetic pigment content in edible halophytes in greenhouse and indoor farming. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1105162

Flowers, T. J., Hajibagheri, M. A., & Clipson, N. J. W. (1986). *Halophytes*. *61*(3), 313–337.

Forlani, G., & Funck, D. (2020). A Specific and Sensitive Enzymatic Assay for the Quantitation of L-Proline. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.582026

García, I., & Mendoza, R. (2014). *Lotus tenuis* seedlings subjected to drought or waterlogging in a saline sodic soil. *Environmental and Experimental Botany*, *98*, 47–55. https://doi.org/10.1016/j.envexpbot.2013.10.002

Gardner, W. H. (1979). *How water moves in the soil. 32*(2), 13–18.

Grigore, M. N., Ivanescu, L., & Toma, C. (2014a). Anatomical Structure in Different Family Species—Lepidium cartilagineum (J.C.Mayer) Thell. Ssp. Crassifolium (Waldst.&Kit.) Thell. In *Halophytes: An Integrative Anatomical Study* (pp. 331–340). Springer International Publishing. https://doi.org/10.1007/978-3-319-05729-3

Grigore, M. N., Ivanescu, L., & Toma, C. (2014b). General morphological and anatomical adaptations in halophytes. In *</i>Halophytes: An integrative anatomical study</i>* (pp. 33–37). Springer Cham Heidelberg New York Dordrecht London.

Grigore, M. N., Villanueva, M., Boscaiu, M., & Vicente, O. (2012). Do Halophytes Really Require Salts for Their Growth and Development? An Experimental Approach. *Notulae Scientia Biologicae*, *4*(2), 23–29. https://doi.org/10.15835/nsb427606

Hackl, P., & Ledolter, J. (2023). A Statistical Analysis of the Water Levels at Lake Neusiedl. *Austrian Journal of Statistics*, *52*(1), 87–100. https://doi.org/10.17713/ajs.v52i1.1444

Hailu, B., & Mehari, H. (2021). Impacts of Soil Salinity/Sodicity on Soil-Water Relations and Plant Growth in Dry Land Areas: A Review. *Journal of Natural Sciences Research*. https://doi.org/10.7176/JNSR/12-3-01

Hansen, J., & Møller, I. (1975). Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Analytical Biochemistry*, *68*(1), 87–94.

Hasanuzzaman, M., Bhuyan, M. H. M. B., Parvin, K., Bhuiyan, T. F., Anee, T. I., Nahar, K., Hossen, M. S., Zulfiqar, F., Alam, M. M., & Fujita, M. (2020). Regulation of ROS Metabolism in Plants under Environmental Stress: A Review of Recent Experimental Evidence. *International Journal of Molecular Sciences*, *21*(22), Article 22. https://doi.org/10.3390/ijms21228695

Häusler, H. (2020). *Wie gelangte das Salz in die Salzlacken? – Zum Stand der Forschung über die Entstehung des „Salzführenden Horizontes“ und der Salzböden des Seewinkels*. *157*, 145–157.

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments. *Plant Signaling & Behavior*, *7*(11), 1456–1466. https://doi.org/10.4161/psb.21949

Herzig, A. (2014). Der Neusiedler See—Limnologie eines Steppensees. *Denisia*, *33*, 101–114.

Hoberg, E., Marschner, P., & Lieberei, R. (2005). Organic acid exudation and pH changes by Gordonia sp. And Pseudomonas fluorescens grown with P adsorbed to goethite. *Microbiological Research*, *160*(2), 177–187. https://doi.org/10.1016/j.micres.2005.01.003

Ismayilov, A. I., Mamedov, A. I., Fujimaki, H., Tsunekawa, A., & Levy, G. J. (2021). Soil Salinity Type Effects on the Relationship between the Electrical Conductivity and Salt Content for 1:5 Soil-to-Water Extract. *Sustainability*, *13*(6), 3395. https://doi.org/10.3390/su13063395

John, B., Sulaiman, C., George, S., & Reddy, V. (2014). Total phenolics and flavonoids in selected medicinal plants from Kerala. *Int. J. Pharm. Pharm. Sci*, *6*(1), 406–408.

Krachler, R. (2007). *Renaturierung von Sodalacken im burgenländischen Seewinkel*. *1*, 23–27.

Krachler, R., Krachler, R., Gülce, F., Keppler, B. K., & Wallner, G. (2018). Uranium concentrations in sediment pore waters of Lake Neusiedl, Austria. *Science of The Total Environment*, *633*, 981–988. https://doi.org/10.1016/j.scitotenv.2018.03.259

Kumari, A., Das, P., Parida, A. K., & Agarwal, P. K. (2015). Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00537

Kumari, A., & Parida, A. K. (2018). Metabolomics and network analysis reveal the potential metabolites and biological pathways involved in salinity tolerance of the halophyte *Salvadora persica*. *Environmental and Experimental Botany*, *148*, 85–99. https://doi.org/10.1016/j.envexpbot.2017.12.021

Laware, S. L. (2015). Sequential extraction of plant metabolites. *International Journal of Current Microbiology and Applied Sciences*, *4*(2), 33–38.

Lepidium cartilagineum—Burgenland Flora. (n.d.). Retrieved 3 September 2024, from https://www.burgenlandflora.at/flora/detail/lepidium-cartilagineum/#c-8

Lichtenthaler, H. K., & Wellburn, A. R. (1983). *Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents*.

Lotus tenuis—Burgenland Flora. (n.d.). Retrieved 3 September 2024, from https://www.burgenlandflora.at/flora/detail/lotus-tenuis/

Mile, O., Mészáros, I., Veres, S., & Lakatos, G. (2002). *Ecophysiological study on the salt tolerance of a pannonian endemism (Lepidium crassifolium (W. et K.)) in inland saline area*. *46*(3–4), 249–250.

Moore, S., & Stein, W. H. (1948). Photometric nin-hydrin method for use in the ehromatography of amino acids. *Journal of Biological Chemistry*, *176*, 367–388.

Murakeözy, É. P., Nagy, Z., Duhazé, C., Bouchereau, A., & Tuba, Z. (2003). Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *Journal of Plant Physiology*, *160*(4), 395–401. https://doi.org/10.1078/0176-1617-00790

Mylona, P., Pawlowski, K., & Bisseling', T. (1995). *Symbiotic Nitrogen Fixation*. *7*(7).

O'Geen, A. T. (2013). *Soil Water Dynamics*. Nature Education Knowledge 4(5):9.

Painuli, S., Quispe, C., Herrera-Bravo, J., Semwal, P., Martorell, M., Almarhoon, Z. M., Seilkhan, A., Ydyrys, A., Rad, J. S., Alshehri, M. M., Daştan, S. D., Taheri, Y., Calina, D., & Cho, W. C. (2022). Nutraceutical Profiling, Bioactive Composition, and Biological Applications of Lepidium sativum L. *Oxidative Medicine and Cellular Longevity*, *2022*, 2910411. https://doi.org/10.1155/2022/2910411

Pizarro, L., & Stange, C. (2009). Light-dependent regulation of carotenoid biosynthesis in plants. *Ciencia e Investigación Agraria*, *36*(2), 143–162. https://doi.org/10.4067/S0718-16202009000200001

Popp, M., & Albert, R. (1980). Freie Aminosäuren und Stickstoffgehalt in Halophyten des Neusiedlersee-Gebietes. *Flora*, *170*(3), 229–239. https://doi.org/10.1016/S0367-2530(17)31208-2

Pungin, A., Lartseva, L., Loskutnikova, V., Shakhov, V., Popova, E., Skrypnik, L., & Krol, O. (2023). Effect of Salinity Stress on Phenolic Compounds and Antioxidant Activity in Halophytes Spergularia marina (L.) Griseb. And Glaux maritima L. Cultured In Vitro. *Plants*, *12*(9), 1905. https://doi.org/10.3390/plants12091905

Qadir, M., Oster, J. D., Schubert, S., Noble, A. D., & Sahrawat, K. L. (2007). Phytoremediation of Sodic and Saline-Sodic Soils. In *Advances in Agronomy* (Vol. 96, pp. 197–247). Academic Press. https://doi.org/10.1016/S0065-2113(07)96006-X

Qasim, M., Abideen, Z., Adnan, M. Y., Gulzar, S., Gul, B., Rasheed, M., & Khan, M. A. (2017). Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. *South African Journal of Botany*, *110*, 240–250. https://doi.org/10.1016/j.sajb.2016.10.005

Qiu, N., Lu, Q., & Lu, C. (2003). Photosynthesis, photosystem II efficiency and the xanthophyll cycle in the salt-adapted halophyte *Atriplex centralasiatica*. *New Phytologist*, *159*(2), 479–486. https://doi.org/10.1046/j.1469-8137.2003.00825.x

Rabhi, M., Castagna, A., Remorini, D., Scattino, C., Smaoui, A., Ranieri, A., & Abdelly, C. (2012). Photosynthetic responses to salinity in two obligate halophytes: *Sesuvium portulacastrum* and

Tecticornia indica. *South African Journal of Botany*, *79*, 39–47. https://doi.org/10.1016/j.sajb.2011.11.007

Rahman, Md. M., Mostofa, M. G., Keya, S. S., Siddiqui, Md. N., Ansary, Md. M. U., Das, A. K., Rahman, Md. A., & Tran, L. S.-P. (2021). Adaptive Mechanisms of Halophytes and Their Potential in Improving Salinity Tolerance in Plants. *International Journal of Molecular Sciences*, *22*(19), 10733. https://doi.org/10.3390/ijms221910733

Ray, A., Kundu, S., Mohapatra, S. S., Sinha, S., Khoshru, B., Keswani, C., & Mitra, D. (2024). An Insight into the Role of Phenolics in Abiotic Stress Tolerance in Plants: Current Perspective for Sustainable Environment. *Journal of Pure and Applied Microbiology*, *18*(1), 64–79. https://doi.org/10.22207/JPAM.18.1.09

Rhoades, J. D., Manteghi, N. A., Shouse, P. J., & Alves, W. J. (1989). Estimating Soil Salinity from Saturated Soil-Paste Electrical Conductivity. *SOIL SCI. SOC. AM. J.*, *53*.

Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., Kim, Y.-O., & Redman, R. S. (2008). Stress tolerance in plants via habitat-adapted symbiosis. *The ISME Journal*, *2*(4), 404–416. https://doi.org/10.1038/ismej.2007.106

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., & Prado, F. E. (2009). Soluble sugars. *Plant Signaling & Behavior*, *4*(5), 388–393. https://doi.org/10.4161/psb.4.5.8294

Saghafi, D., Delangiz, N., Lajayer, B. A., & Ghorbanpour, M. (2019). An overview on improvement of crop productivity in saline soils by halotolerant and halophilic PGPRs. *3 Biotech*, *9*(7), 261. https://doi.org/10.1007/s13205-019-1799-0

Sannazzaro, A. I., Bergottini, V. M., Paz, R. C., Castagno, L. N., Menéndez, A. B., Ruiz, O. A., Pieckenstain, F. L., & Estrella, M. J. (2011). Comparative symbiotic performance of native rhizobia of the Flooding Pampa and strains currently used for inoculating Lotus tenuis in this region. *Antonie van Leeuwenhoek*, *99*(2), 371–379. https://doi.org/10.1007/s10482-010-9502-9

Sharma, R. C., Saxena, R. K., & Verma, K. S. (2000). *Reconnaissance mapping and management of salt-affected soils using satellite images: International Journal of Remote Sensing: Vol 21, No 17*. *21*(17), 3209–3218. https://doi.org/10.1080/014311600750019831

Simon, E. M., Mburu, M. W., & Koskei, R. K. (2024). *The Impact of Varying Stages of Maturity on the Chemical Composition and Antioxidant Activity of Yacon (Smallanthus sonchifolius) Leafy Vegetable*. https://doi.org/10.24018/ejfood.2024.6.3.782

Soja, G., Züger, J., Knoflacher, M., Kinner, P., & Soja, A.-M. (2013). Climate impacts on water balance of a shallow steppe lake in Eastern Austria (Lake Neusiedl). *Journal of Hydrology*, *480*, 115–124. https://doi.org/10.1016/j.jhydrol.2012.12.013

Stavi, I., Thevs, N., & Priori, S. (2021). Soil Salinity and Sodicity in Drylands: A Review of Causes, Effects, Monitoring, and Restoration Measures. *Frontiers in Environmental Science*, *9*, 712831. https://doi.org/10.3389/fenvs.2021.712831

Stroh, P. A., Humphrey, T. A., Burkmar, R. J., Pescott, O. L., Roy, D. B., & Walker, K. J. (2023). *Lotus tenuis Waldst. & Kit. Ex Willd. In BSBI Online Plant Atlas 2020*. https://plantatlas2020.org/atlas/2cd4p9h.8tq

Su, F., Zhao, B., Dhondt-Cordelier, S., & Vaillant-Gaveau, N. (2024). Plant-Growth-Promoting Rhizobacteria Modulate Carbohydrate Metabolism in Connection with Host Plant Defense Mechanism. *International Journal of Molecular Sciences*, *25*(3), 1465. https://doi.org/10.3390/ijms25031465

Teakle, N., Flowers, T., Real, D., & Colmer, T. (2007). Lotus tenuis tolerates the interactive effects of salinity and waterlogging by 'excluding' Na+ and Cl- from the xylem. *Journal of Experimental Botany*, *58*(8), 2169–2180. https://doi.org/10.1093/jxb/erm102

Teakle, N. L., Real, D., & Colmer, T. D. (2006). Growth and ion relations in response to combined salinity and waterlogging in the perennial forage legumes Lotus corniculatus and Lotus tenuis. *Plant and Soil*, *289*(1–2), 369–383. https://doi.org/10.1007/s11104-006-9146-8

United Nations, D. of E. and S. A., Population Division. (2022). *World Population Prospects 2022: Ten Key Messages*. https://population.un.org/wpp/Publications/

Wang, X., Chang, L., Wang, B., Wang, D., Li, P., Wang, L., Yi, X., Huang, Q., Peng, M., & Guo, A. (2013). Comparative Proteomics of Thellungiella halophila Leaves from Plants Subjected to Salinity Reveals the Importance of Chloroplastic Starch and Soluble Sugars in Halophyte Salt Tolerance *. *Molecular & Cellular Proteomics*, *12*(8), 2174–2195. https://doi.org/10.1074/mcp.M112.022475

Wang, Y., Zhang, Z., Zhang, P., Cao, Y., Hu, T., & Yang, P. (2016). Rhizobium symbiosis contribution to short-term salt stress tolerance in alfalfa (Medicago sativa L.). *Plant and Soil*, *402*(1–2), 247–261. https://doi.org/10.1007/s11104-016-2792-6

Warrence, N. J., Bauder, J. W., & Pearson, K. E. (2002). *Basics of salinity and sodicity effects on soil physical properties* (129; pp. 1–29). Department of Land Resources and Environmental Sciences, Montana State University-Bozeman.

Weimberg, R. (1987). Solute adjustments in leaves of two species of wheat at two different stages of growth in response to salinity. *Physiologia Plantarum*, *70*(3), 381–388. https://doi.org/10.1111/j.1399-3054.1987.tb02832.x

Xu, X., Huang, G., Sun, C., Pereira, L. S., Ramos, T. B., Huang, Q., & Hao, Y. (2013). Assessing the effects of water table depth on water use, soil salinity and wheat yield: Searching for a target depth for irrigated areas in the upper Yellow River basin. *Agricultural Water Management*, *125*, 46–60. https://doi.org/10.1016/j.agwat.2013.04.004

Yan, S., Chong, P., & Zhao, M. (2022). Effect of salt stress on the photosynthetic characteristics and endogenous hormones, and: A comprehensive evaluation of salt tolerance in Reaumuria soongorica seedlings. *Plant Signaling & Behavior*, *17*(1), 2031782. https://doi.org/10.1080/15592324.2022.2031782

ZAMG, n. d. (n.d.). *Jahrbuch—ZAMG*. Retrieved 29 August 2024, from https://www.zamg.ac.at/cms/de/klima/klimauebersichten/jahrbuch

Zhang, H., Han, B., Wang, T., Chen, S., Li, H., Zhang, Y., & Dai, S. (2012). Mechanisms of Plant Salt Response: Insights from Proteomics. *Journal of Proteome Research*, *11*(1), 49–67. https://doi.org/10.1021/pr200861w

Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C., & Wang, P. (2021). Regulation of Plant Responses to Salt Stress. *International Journal of Molecular Sciences*, *22*(9), 4609. https://doi.org/10.3390/ijms22094609

Zhu, J.-K. (2002). SALTAND DROUGHT STRESS SIGNAL TRANSDUCTIONIN PLANTS. *Annual Review of Plant Biology*, *53*(1), 247–273. https://doi.org/10.1146/annurev.arplant.53.091401.143329

Zimmermann-Timm, H., & Teubner, K. (2021). *3.8 Folgen der Grundwassersenkung am Beispiel des*.