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Lecture summaries

Frederick Zittrell

Allelopathy

Functions, Release, Interactions and Current Research Approaches *(Fernandez)*

Definition (Inderjit et al., 2011):

Suppression of the growth and/or establishment of neighboring plants by chemicals released from a plant or plant parts.

 \rightarrow Allelochemicals: Secondary compounds of plant origin that interact with their environment and possess allelopathic activities.

Other definitions also include stimulative effects, reciprocal interactions, for example with microorganisms, and autotoxicity.

Characteristics of allelochemicals

- Present in all organs (root, shoot, leave, flower, fruit)
- Variable in metabolic pathway of production
- Different ways of release: Volatilization (into the air), leaching (solute into the ground), active exudation and decomposition
- Variable basis of detrimental effects on growth (enzyme activity, cell division, growth regulator activity, membrane permeability...)

Methodology of Allelopathy Research

General difficulty: Separation of competition and allelopathic effects.

- Preliminary studies: Identification and characterization of allelochemicals (chemical properties, metabolical and anatomical derivation, way of release, variability in time...)
- Common approach: Filter paper bioassays; addition of macerated, presumably allelochemicals containing organs of a donor plant in different developmental stages and concentrations on seedlings of target species → Detection of allelopathic potentiality of respective plant organ in respective plant community
- Field experiments: Direct addition of suspected substance onto the field





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Example 2: Allelopathic impact on secondary succession in Mediterranean plant communities; Bioassay approach

1. Application of needle and root macerates of 10, 5 and 2.5% concentration from *Pinus halepensis* of 10, 20 and 30y age on seedlings from 2 herb species.

Result: Significant decrease of germination rate in any bioassay combination; needles of young pines and roots of old pines inhibit growth.

2. Application of needle macerates of young *P. halepensis* on 15 target species put in natural and sterilized soils.

Result: Inhibition of twice as many species on sterilized (80%) than on natural (40%) soils \rightarrow Microorganisms greatly modulate allelopathic potential of young *P. halepensis.*

Furthermore, macerates promote microbial activity (cause to be investigated).

3. Application of needle and root macerates of old *P. halepensis* on same species' seedlings, put in natural and sterilized soils.

Result: Decreased germination speed and growth of seedlings \rightarrow autotoxicity; inhibition of pine regeneration in old pine wood, oaks are not affected

 \rightarrow *P. halepensis* is a driver of secondary succession



Example 3: Allelopathy in the ecosystem forest

Research on downy oak forest (Mediterranean) with understory of a high cover of *Cotinus coggygria* (smoke tree); biodiversity changes between *C. coggygria* absent and present were observed.

- 1. Chemical analysis: High diversity of terpenes in smoke tree leaves of all developmental stages, especially in litter
- 2. Bioassay on plant diversity: Green and senescent leaves decrease growth of seedlings (from more or less sensitive species); increased allelopathic effect in sterilized soil
- 3. Bioassay on oak regeneration: Young oaks are sensitive to *C. coggygria* allelochemicals
- → Cotinus coggygria has a strong allelochemical influence on biodiversity

The "Novel Weapons" Hypothesis

Theory about the success of invasive plants: In natural environment plants co-evolve – defense against allelochemicals develops slowly along with them. If plants invade a very different ecosystem, the native plants have no sufficient defense against the differently evolved allelochemicals, the "novel weapons", and are likely to lose the battle for growth and reproduction.

Microorganisms as Targets and Mediators of Allelopathy

For example: *Alliaria petiolata* releases allelochemicals inhibiting mychorrhiza-fungi, thereby decreasing the fitness of plant competitors that rely on mychorrhizae.



Diverse sites of microbial interaction (Cippolini et al., J.Chem Ecol 2012)

Microorganisms play different roles



Inderjit et al., 2011

They can be harmed by allelochemicals, therefore neutralize them. In this process, even more toxic compounds (for plants) could be produced, thus enhancing allelopathic effects on plants. Mychorrhizal networks can facilitate the transfer of allelochemicals.

Impact of Plant Communities Change on Leaf Litter Decomposition

(Baldy)

With the oak observatory in southern France (Mediterranean area), experimental long-term studies on the effect of decreasing rainfall due to climate change on *Quercus* pubescens forest diversity and functioning can be conducted.

Q. pubescens is the dominant tree species in France, the observed oak-forest of 95 hectare, which has not been used for 70 years, is studied using a 40 meters long crossed gateway system on two levels, allowing monitoring from soil to canopy without disturbance of the ecosystem.

Example 1: Impact of climate change

Prognosis till 2100: 30% decrease of rainfall and doubling of the summer dry period

 \rightarrow increased water stress

Experimental approach: Covering of a part of the forest during rainfall (ongoing study)

Example 2: Litter-mixing effects of decomposition

Hypothesis: Plant diversity $\uparrow \rightarrow$ chemical diversity \uparrow of litter \rightarrow taxonomic and functional diversity \uparrow of soil microbes \rightarrow efficiency \uparrow of litter decomposition

Experimental approach: One-year decomposition experiment with mixtures of one to four different species (*Acer monspessulanum, Quercus pubescens, Cotinus coggygria, Pinus halepensis*) in litter-cages put on the forest soil.

Results:

- Positive effect of *A. monspessulanum* on decomposition efficiency in any two-speciesmixture.
- Positive effect of *P. halepensis* on decomposition efficiency in any mixture, increasing with raising number of other species.
- Oppositional effect of *P. halepensis* mixed with *Q. pubescens*; *Q.* favoring *P.* decomposition, *P.* inhibiting *Q.* decomposition.

 \rightarrow Hypothesis partly certified – biodiversity does not promote litter decomposition per se, but depends on the different species and their combination.

Acidification of Freshwaters and Soils

Causes and Consequences

(Baldy)

19th century: First industrial revolution with coal, steam engines and railway



20th century: Second industrial revolution with oil, combustion engines, motorization etc.

World energy consumption per year (ourfiniteworld.com)

 NO_x^{+} and SO_2^{+} emissions:

Natural	Anthropogenic	
Volcanic events ^{*+} Forest fires [*]	Industry [*] Transport ^{*+}	Chemical transformation into strong acids H_2SO_4 and HNO_3 \rightarrow Acidification of soil
Decomposition ^{*+} Oceans ⁺	Agriculture⁺ …	

The problem: Acid-cations exchange with nutrient cations bound to soil particles \rightarrow leaching of nutrients; self-amplifying effect.

Countermeasure: Calco-magnesium liming, leading to changes in soil pH, Ca-Mg-content and humus morphology – but depends on geological substrates: it is beneficial to sandstone, but detrimental to granite.

Botanical practical work; theoretical background

Water potential

Jiří Mastný

Water potential is the chemical potential of water in a specified part of the system, compared with the chemical potential of pure water at the same temperature and atmospheric pressure. Water potential is measured in units of pressure MPa. It was defined, that the water potential of pure, free water at atmospheric pressure and at a temperature of 298 K is 0 MPa.

Plants retain in biomass less than 1% of the water absorbed. Most of the water is lost by transpiration, which is the evaporation of water from plants. This is necessary for photosynthesis, because the stomates, which allow CO_2 to enter the leaf, also caused water loss. There is a gradient in water potential in the plants. Highest (less negative) water potentials occur in the soil and lowest water potentials are usually at the top of the plant. This is water potential in plants during the day in optimal condition. However, there are some changes at night. At night the gradient collapses or gets very small because there is little transpiration.

Water potential response also when are dry conditions in soil. Water potential of leafs decrease at dry conditions in soil. Thus lead to hormonal signals (mainly abscisic acid) and stomata close.

Long distance movement of water in the plant occurs by bulk flow. Water in the xylem is under tension. The source of tension in the xylem is the negative pressure that develops in the walls of mesophyll cells when water evaporates by stomata.

The cell wall is something like a very fine capillary wick soaked with water. Surface tension in the crevices of the wall induces a negative pressure in the water. Thus the motive force for xylem transport of water is generated at the air-water interfaces within the leaf by transpiration.

Protoplasma in cells contain 85-90% water. Live cells must maintain a positive hydrostatic pressure (remain turgid) to be physiologically active. Turgor is needed for cell expansion and structural rigidity of non-lignified organs like leaves.

In roots, water moves by diffusion through cell membranes. The driving force is the difference in water potential. In the xylem water moves by bulk flow and the driving force is the difference in pressure (ψ_p). From the leaf air spaces to the atmosphere near the leaf surface water vapour moves by diffusion (no membrane), so the driving force is only the difference in water vapour concentration.

$$\psi_{\rm w} = \psi_{\pi} + \psi_p$$

Water potential in any part of the system is the sum of the osmotic potential (ψ_{π}) and the hydrostatic pressure (ψ_p)

Hydraulic lift is the movement of water from deep moist soils to drier surface soils through the root system. This occurs primarily at night, when the plant is at equilibrium with root water potential.

CAM plants are adapted to dry condition – these plants have maximum stomatal conductance at night, when is lower air temperature. This also means that hydraulic lift occurs during the day in the CAM plant.

Reference: Lambers H., 2008 Plant Physiological Ecology - Second Edition, Springer ISBN: 978-0-387-78340-6

Plant - Water Relations

Alena Nosova

Although water is the most abundant molecule on the Earth's surface, the availability of water is the factor that most strongly restricts terrestrial plant production on a global scale. Thus, if we want to explain natural patterns of productivity or to increase productivity of agriculture or forestry, it is crucial that we understand the controls over plant water relations and the consequences for plant growth of an inadequate water supply.

The water in the plant cell

The water in the plant cell occurs in several forms:

<u>Water of hydratation</u> (5-10% total cell water) - associated with ions, dissolved organic substances and macromolecules. Because of dipole character, water molecules are able to associate with ions, form several layers of structured water and cover the macromolecules with thin cove of water. Electrostatic forces between ions and water molecules. Most of the water of hydration is bound by capillary forces in the protoplasm and cell wall. The forces holding water on the surface of structural elements in a matrix can be expressed in terms of the matric pressure T.

<u>Stored water</u> – water in solutions, reserved in specialized cell compartments, most easily translocated. The osmotic pressure π of a solution is given by:

$$\pi^* = \mathbf{n} \cdot \mathbf{R} \cdot \mathbf{T} = 2 \cdot 27 \cdot \mathbf{n} \frac{\mathbf{T}}{273} \text{ [MPa]}$$

 π increases with absolute temperature T and with the number of dissolved particles n. For example, during the conversion of sugars to starch and revers process, plant cell can rapidly alter its osmotic pressure and regulate net water influx.

<u>Interstitial (vascular) water</u> is a transport medium in the spaces between cells and in the conducting elements of the xylem and phloem systems.

The water potential of plant cells

It is the thermodynamic state of the water rather than its total quantity that influences the biochemical activity of protoplasm. The thermodynamic state of the water in a cell can be described as a water potential:

$$\Psi_{\text{cell}} = (-)\Psi_{\pi} + (+)\Psi_{\text{P}}$$

Water potential is the chemical potential of water in a specified part of the system, compared with the chemical potential of pure water at the same temperature and atmospheric pressure. Water potential is measured in units of pressure MPa. It was defined, that the water potential of pure, free water at atmospheric pressure and at a temperature of 298 K is 0 MPa.

The osmotic potential $\Psi\pi$ is invariably negative, whereas the pressure potential Ψp can be positive, zero or in exceptional cases even negative. A negative water potential indicates that the cell as a whole is under tension. Despite that matric potential $\Psi\tau$ is usually small, sometime it is important to include it in calculations of cell water potential.

When water-saturated, the protoplast has attained its greatest volume and exerts the greatest pressure on the call wall. Due to the internal (turgor) pressure the cell wall is maximally distended. Then the resulting wall pressure compensates for the osmotic effect of the of the cell sap so that net water uptake into the cell is stopped. At this point of water saturation Ψ cell = 0 and $\Psi\pi = \Psi p$. Loss of water leads to reduction of the vacuolar volume and a rise in cell sap concentration. Increasingly pressure is exerted on the protoplast by the cell wall, until the cell volume has diminished to a threshold value, beyond which the cell wall can shrink no further (zero turgor point). If the cell is in an aqueous (hypertonic) medium (Fig.1,2) the protoplast begins to pull away from the cell wall, this stage is called incipient plasmolysis ($\Psi p = 0$, Ψ cell = $\Psi\pi$).



Figure 1. Relative position of the plasma membrane for a plant cell undergoing plasmolysis: (a) turgid cell with the plasma membrane pushing against the cell wall; (b) cell just undergoing plasmolysis, i.e., at incipient plasmolysis; and (c) cell with extensive plasmolysis, as the plasma membrane has pulled away from the cell wall over a large region.



Figure 2. Water-potential diagram for vacuolated cells in a hypertonic medium (a).

Water relations of the whole plant

The shoot of terrestrial plants steadily loses water to the air surrounding it, this water must be replaced from the soil. Transpiration, water uptake and conduction of water from the roots to the transpiring surfaces are inseparably linked processes in water balance.

Water uptake by roots

A plant can withdraw water from the soil only as long as the water potential of its roots is more negative than of soil solution around. The rate of water uptake by roots increases in parallel with increase of absorbing surface of the root system.

Roots usually develop negative water potentials of a few tenths MPa, which is nevertheless quite sufficient to withdraw the greater part of the capillary water from most soils. Plants can obtain more water from the soil by actively lowering their root potential: hygrophytes -1MPa, crop plants from -1 to -2Mpa, mesophytes to -4MPa and xerophytes at most -6 MPa.

During the night, water drawn from the deep, moist soil horizon is conveyed not only to the shoots but also to the roots nearer the surface (Fig.3). If the water potential in the uppermost soil layers is lower than that of these roots the latter lose water to the surrounding soil. This nocturnal hydraulic lift in deep-rooting plants helps other plants with more superficial root systems to survive period of drought.



Figure 3. Hydraulic lift in the root system of Artemisia tridentate (Dawson, 1993)

Soil-plant-air gradient of water potential is a basis of water flux inside the plants

The plant bridges the steep water-potential gradient between soil and air (Fig.4). Because the shoot is exposed to the vapour-pressure deficit of the air a flow of water through the plant is set in motion. The steepest water-potential gradient is that between the shoot surface and dry air. Resistance to water transport through soil-plant-air decrease.



Figure 3 Water-potential gradients and resistances to water transport between soil, plant and atmosphere.

The potential gradient in the soil-plant-atmosphere continuum is the driving force for water transport through the plant. The water potential Ψz at a particular location in the plant is lower, the lower the water potential in the soil, the greater the effect of gravity Ψg , the greater the hydraulic resistances r_j , between the soil and the point of reference z in the shoot, and the more water flowing through the plant (sum of partial fluxes Σ_{ij}):

$$\Psi_{z} = \Psi_{soil} + \Psi_{g} + \Sigma_{soil}^{z} j_{i} \cdot r_{i}$$

The plant would be expected to exhibit a speed gradient in water potential only when large quantities of water are flowing through it, i.e. when conditions promote intensive transpiration.

The path of water in the plant

Within the plant water moves from cell to cell (short distance transport) and through xylem (long distance transport).

Cell to cell diffusion is based on hydrostatic gradient between the cells and apoplastic translocation.

In the root the water passes parenchyma, endodermus (with blocked by Casparian strips apoplastic transport) and then goes to central stele where the long distance transport takes place.

The rate of sap flow

The amount of water moved through the vascular system in unit time is dependent on the relative conducting area (ratio of xylem conducting area A_{xyl} and the leaf surface area Al or leaf mass of the transpiring parts that are supplied by this conducting tissue) and flow resistances, on the physiological state of the plant and on environmental conditions.

Water Loss from the Plants

Plants lose the water in vapour from by evaporation (transpiration) and in liquid form (guttation, negligible contribution).

Evaporation from the water surface depends on water-vapour content and temperature of air.

Evaporation under conditions of unlimited water supply and unimpeded removal of the water vapour is called the potential evaporation. The actual evaporation from moist surfaces is usually less than potential evaporation, because water is almost never replenished as rapidly as is lost.

Transpiration

Transpiration is an inevitable consequence of photosynthesis; however, it also has important direct effects on the plant because it is a major component of the leaf's energy balance.

Within the organs of the plant, water evaporates from the intercellular air space (liquidvapour conversion) and escapes through the stomata. After, vapour diffuses into boundary layer and thence into the open air. Transpiration is affected by external factors to the extent to which they alter the steepness of the vapour pressure gradient between the plant surface and air. Dryness and temperature of the air, warming of the leaf surface (by irradiation) also leads the transpiration even with high air humidity. For big leaves wind can remove the vapour-saturated layer and effect on transpiration.

Maximal transpiration

Is a unimpeded intensity of evaporation under regular conditions of evaporation in natural habitat. The rate of maximal transpiration is connected with morphology and life-form of a plant. The highest transpiration intensities have been recorded for tall herbs of the meadows on river banks in Asia and for floating and swamp plants.

Transpiration as a diffusion process, regulation of transpiration

Diffusion of the water through the leaf can be described by resistances (conductances) on the different steps (Fig.5,A):

$g_{\text{leaf}} = g_s + g_{\text{cut}}$

 g_{s} – stomatal conductance is determined by anatomical features, arrangement and density of the stomata.

 g_{cut} – cuticular conductance, is so low that it can be ignored in most cases, except when the stomatal conductance is extremely low.

Transpiration is strictly dependent on the physical conditions affecting evaporation only as long as degree of stomatal opening does not change. Plants can regulate stomatal opening and quantitative changes can be determined porometrically. A temporary reduction in the degree of stomatal opening elicited by a decrease in light intensity, dry air, water deficit, extremes temperature and toxic gases. The way stomata respond may differ from one plant species to another and even from one individual to the next within the same species. Even the leaves of a single plant vary quite considerably in this respect, depending on their age, the conditions under which they developed and their positions on the shoot.

The water balance of a plant

The basic processes involved in the water balance of a plant are water uptake, conduction and water loss. The difference between water absorption and transpiration, i.e. water balance indicates the status of water equilibrium in the plant: negative balance means that the uptake is insufficient to meet the requirements of transpiration, and positive values means that transpiration is decreased while uptake is unchanged. Short-term fluctuations (Fig.5) if water balance reflects the interplay of the various water-regulating mechanisms (mostly changes in stomata aperture) and occurs during the day. Long-term (seasonal) fluctuations are based on changes of rainy/dry periods during the year.



Figure 5. Time course of the leaf conductance to water vapour (A, B), the relative water content (RWC) of the leaves (C, D), and the leaf water potential (E, F) for two Mediterranean tree species, the relatively drought-tolerant Olea oleaster (olive) and the less tolerant Laurus nobilis (laurel). RWC is defined as the amount of water per unit plant mass relative to the amount when the tissue is fully hydrated. Measurements were made in dry season.

Indicators of the state of water balance

Water balance can be computed from quantitative determinations of water uptake and transpiration, therefore it is customary to make an indirect estimate of the water balance through its effect upon the water content or water potential of the plant.

<u>Relative water content (RWC)</u>, the water content at any particular time of observation helps to demonstrate the water deficit (Fig.5):

RWC = Wact/Wsat *100%

The actual water content (Wact)

Water content of the leaves under conditions of saturation (Wsat)

Water saturation deficite (WSD) shows the water deficiency:

WSD = (Wsat-Wact)/Wsat *100%

Fluctuations in water content affect the concentration of the cell sap and the turgor of the cells. The osmotic potential rises when the water balance is negative (osmoregulating processes should be taken into account as well). As an indicator for the state of the water balance the actual value of osmotic potential is compared with its optimum value, and the osmotic maximum under conditions of extreme water shortage.

Water relations in the different plant types

<u>Hydrostable plants</u>: shows a great sensitivity of stomata respond; store the water in roots and the wood and bark of the stems or trunks. Include aquatic plants, succulents, sciophytes, certain grasses and trees of humid regions.

<u>Hydrolabile plants</u> can afford to risk quite large losses of water and the consequent rise in cell sap concentration. Such plants can tolerate strong fluctuations in water potential. Many herbs of sunny habitats, steppe grasses, woody plants, pioneer species.

Conclusion

The shoot of terrestrial plants steadily loses water to the air surrounding it, this water must be replaced from the soil. Transpiration, water uptake and conduction of water from the root to the transpiring surfaces are inseparably linked processes in water balance. The vapour-pressure deficit of the air is the driving force for evaporation, and the water in the soil is crucial quantity in water supply. The water balance is maintained by a continuous flow of water, and is thus in a state of dynamic equilibrium.

References:

Larcher W., 1995 Physiological Plant Ecology - Third Edition, p.217-254

Lambers H., 2008 Plant Physiological Ecology - Second Edition, Springer ISBN: 978-0-387-78340-6

Practical work: turgor potential of a potato

Frederick Zittrell

In the first part, students should learn about turgor pressure by conducting a simple experiment.

In order to obtain physical stability, plant cells actively store lons, sugars and other organic compounds in their vacuole, producing high molarity of this solution. The physical pressure of the vacuole is antagonized by the cell wall, leading to high tension against it – this is called turgor; it is the only mechanism for non-lignified plant tissues to produce stability.

Depending on the molarity of the solution in the vacuole and the surrounding solution, turgor pressure can be higher or lower. Students were to determine the turgor potential (the higher the molarity of the vacuole, the lower the potential) of potato parenchyma with a simple experiment.

Small pieces of potato were each put in solutions of different molarity, starting at 0M (distilled water) up to 1M (sucrose solution) for 1 hour. By weighing the pieces before and after accurately, it could be calculated whether they gained or lost weight during the treatment.

In hypotonic environment (0M), water diffuses into plant cells establishing turgor; vice versa, in hypertonic solutions plant cells lose water.

Thus, a diagram with the measured change in weight in percent depending on the molarity of the solution is expected to look somehow like this:



Where the fitted curve and x-axis meet is where one can read the molarity of the tissue because water influx and efflux are equal. The turgor potential equals the water potential of a sucrose solution of this molarity.

However, the obtained diagram looks different:



The values do not follow a linear pattern; a failure of experiment execution is as obvious as it is unbelievable. Fitting a curve is not a reasonable option; the turgor potential cannot be determined.

The experiment was conducted in two steps by two different groups of students; it is probable, yet not reconstructable, that the cups that contained the different solutions got mixed up due to non-comprehensible labeling.

In the second part, students were shown how water potential can be measured. The mode of operation of a common pressure chamber, a schematic shown in the following figure, was professionally explained by a highly qualified supervisor.



Pressure chamber (plantstress.com)

The pressure on the leaves within the chamber rises step-wise. The scientist observes the cut surface of the shoot; when xylem sap leaks out, the negative air pressure in the chamber equals the water potential of the leaves.

Zoology practical work

Jiří Mastný

Zoology practical work started at 10 30. First part of lesson was theoretical. First of all, we discussed what the animals are. We defined animals like multicellular, mobile, heterotrophic organisms and we postulated that unicellular animals are not considered as animals. Then the teacher told to us about body plans and biology of animals and about the evolution.

The practical work was based on determining of insects samples. These samples were collected in May. It was necessary to have some basic knowledge about the biology of these animals, before we started determining of these animals. The teacher talked about general introduction in the biology of animals. We obtained determination key. This determination key was made by famous English author Chinery (1993) and the name of this key is "Collins Field Guide Insects of Britain and Northern Europe". First step in determining of animals is divining into groups according to the number of legs or pairs of the legs. There are some examples, what we should know, when we wanted to determine arthropods: Arthropods have thorax and abdomen and they have also lens eyes. Scorpions and spiders have 4 pairs of legs. However scorpions are not so important in Estonian region. Also predatory and orepathic (vegetarian) mites have 4 pairs of legs. Woodlays (crustaceans) have 7 pairs of legs. Hexapods are divided in 3 groups: Springtails, beetles and bugs. Difference between beetles and bugs is that beetles have chawing mouth part, meanwhile bugs have sucking advice.

Then we obtained our samples. First step was split animals from each other and divided them into groups according to determination key. Sometimes it was difficult because of missing legs or strange shape of animal, but most of the samples was quite easy to determine. My group found quite many spiders, diptera and ants. When we finished our work, we collected data from all groups and gave them to Petr Kotas and Martina Balzarová. Petr and Martina put all data together and found the most abundant groups. They presented their findings later afternoon. These findings were quite similar to results of me group.

There were three presentations which started at 16 00.

Jiří Mastný, Jana Baxová and Zdeněk Žampach had presentation about study made by Zak and Gelbrecht (2007). The theme of this study and also theme of this presentation was "The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of fen rewetting". In the first part of presentation they explained basic terms and knowledge about peatland. Then they presented introduction to the paper and results of this study. Results of study show that P, OC and ammonium mobilisation processes on the degree of peat decomposition. Conclusion of this paper is that removal of the highly decomposed peat layer is recommended to reduce the high mobilisation. However, students discussed with Tomáš Picek about this conclusion and they disagreed because this study is only short-term study, but peatlands restoration needs long-term time scale for water table stabilization and ecosystem recovery.