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Autecological study of selected Cretaceous plants using stable carbon isotopes

Studium autekologie vybraných taxonů křídových rostlin pomocí izotopů uhlíku

Master's thesis
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Abstrakt

Tato práce se zabývá analýzou fosilních rostlin z cenomanských Perucko-korycanských souvrství České křídové pánve a Bückebergským souvrstvím z Dolnosaské pánve v Německu. Na základě dřívějších studií je známo, že obě lokality poskytují fluviální sedimenty, které byly utvářeny v oblastech ovlivněných příliv-odlivovými cykly. Mezi fosilními vzorky jsou zastoupeny listy skupiny Ginkgoales – *Eretmophyllum*, *Tritaenia*, větve jehličnanu *Frenelopsis* a také listy lauroidních angiosperm – *Eucalyptolaurus*. Za halofytní rostliny jsou považovány *Frenelopsis*, *Eretmophyllum* a *Tritaenia*, zatímco lauroidní angiospermy jsou považovány za sladkomilnější druhy.

Hlavní použitá metodika byla kutikulární analýza, analýza stabilních izotopů z Bulk (celkového uhlíku) a analýza stabilních izotopů listových vosků. Vzorky byly pozorovány makroskopicky a mikroskopicky pod světelným mikroskopem a skenovacím elektronovým mikroskopem.

Stejnými metodami byly zkoumány jak fosilní, tak recentní vzorky. Pro nalezení podmínek prostředí byly navzorkovány a následně využity recentní rostliny pocházející ze slaných marší ve Velké Británii. Interpretace podporují hypotézu modelu založeného na sedimentologických datech, tedy prostředí slaných marší.

Frenelopsis je interpretován jako slanomilná rostlina, rostl v prostředích s nízkou dostupností vody a vysokou salinitou, stejně jako současná *Halimione portulacoides.*

Pseudoaterophyllites je interpretován jako rostlina rostoucí v prostředí, které dnes obývá *Salicornia.* Jsou to pionýrské zóny, kde salinita kolísá. Zároveň ale tyto druhy vydrží i vysušení a velmi vysokou salinitu. Mají nezvyklou schopnost pufrovat výkyvy prostředí, a proto se vyskytují na celé slané marši.

Eretmophyllum je strom, jehož nároky na růst jsou interpretovány jako velmi suché a s vysokou salinitou. Obýval podobná místa jako *Frenelopsis.*

Lauroidní angiospermy rostly ve vlhkých podmínkách na březích příliv-odlivových kanálů nebo i březích řek. Tyto oblasti se vyskytují ve střední až vysoké marši. Další možná interpretace naměřených dat je model mangrovových porostů, kdy by lauroidní angiospermy rostly přímo v mořem zaplavené části mangrove, kde je vysoká dostupnost vody a nižší salinita oproti vysušovaným částem mangrovů vzdálenějších od pobřeží.

Tritaenia z dolnosaské pánve v Německu je interpretována jako sladkomilnější rostlina, která rostla v podobných podmínkách jako lauroidní angiospermy – nejvyšší marše a břehy vodních toků – nicméně může růst i v jemně zasolených oblastech. V alternativní interpretaci lauroidních angiosperm by rostla společně s nimi v břežní části mangrovů.

Při interpretaci naměřených dat byla uvažována možná zonace, ale spíše než zonace se z recentních dat ukázalo, že je možné interpretovat podmínky prostředí. Data získaná izotopovou analýzou listových vosků přinesla další informace o podmínkách růstu rostlin ve fosilním ekosystému. Tato metoda je vhodná pro další paleoekologické rekonstrukce.

Klíčová slova: Lauroidní angiospermy, analýza stabilních izotopů uhlíku, Ginkgoales, paleoenvironmentální rekonstrukce, Pecínov, Horoušany, Křída, Hloubětín, kutikuly, slané marše, Malá Chuchle, fosilní listy, průduch, halofyty, mangrove, Duingen, Everglades, Bulk analýza, CSIA

Abstract

This thesis presents an analysis of fossil plants from the Cenomanian Peruc-Korycany Formation of the Bohemian Cretaceous Basin and from the Bückeberg Formation of the Lower Saxony Basin in Germany. Based on earlier studies, both areas provide sediments that are considered to have developed in tidally influenced fluvial systems. Studied fossil plants are represented by ginkgoalean plant leaves (*Ertemophyllum*, *Tritaenia*), branches of conifers (*Frenelopsis*) and lauroid angiosperms (*Eucalyptolaurus*). *Frenelopsis*, *Eretmophyllum* and *Tritaenia* are considered to be halophytic plants, while Lauroid angiosperms were considered to grow in fresh water conditions.

The fossil plants were studied using cuticle analysis and two methods of stable carbon isotope analysis: Bulk carbon isotope analysis and Compound Specific Isotope analysis. For cuticle analysis samples were observed and documented macroscopically and microscopically.

To specify the environmental conditions, recent samples from three salt marshes in Great Britain were studied and analysed using the same methods as the fossil samples.

The data from all observations and measurements were processed and their interpretation supported the modelled environment based on the sedimentological data.

Frenelopsis were growing in a haline environment with low water availability, like today's *Halimione portulacoides.*

Pseudoaterophyllites grew in localities similar to today's localities of *Salicornia*, the pioneer zone of the salt marsh and microhabitats with both low and high water availability. It is interpreted as a plant with a good buffering ability which can grow in many various microhabitats.

Eretmophyllum is a tree which grew in a haline environment with low water availability and low change of salinity. It occupied similar habitats as *Frenelopsis.* Lauriod angiosperms grew in conditions of high water availability on the edges of river system, higher on the saltmarsh. As an alternative hypothesis of Lauroid angiosperms a model of mangrove-like environment is suggested, where the Lauroid angiosperms would grow directly on the sea shore. There, water availability is high in comparison with the more saline environments farther away from the sea shore.

Tritaenia from the Lower Saxony Basin in Germany is suggested to have grown in more fresh water ecosystems with good water availability. *Triteania* occupied the high marsh and was slightly salt tolerant.

Zonality of the salt marsh has been taken into account, but rather than precise zonation, the environmental conditions were specified for each studied fossil plant.

The data from the Compound Specific Isotopic Analysis brought further information about the environmental conditions and the method can be used as a new tool in palaeoreconstructions.

Key words: Lauriod angiosperms, carbon stable isotopes analysis, Ginkgoales, palaeoenviromental reconstruction, Pecínov, Horoušany, Cretaceous, Hloubětín, cuticles, salt marshes, Malá Chuchle, fossil leaves, stoma, halophytes, mangroves, Duingen, Everglades, salt tolerant plants, Bulk analysis, CSIA – Compound Specific Isotope Analysis

1 Introduction

The existence of the Bohemian Cretaceous basin is well known and has been extensively described in the literature (see Uličný et al. (1996), Nguyen Tu et al. (2002) etc.). Present models of vegetation are based on available palaeobotanical data. Alternative approaches are needed to specify past environmental conditions. A more detailed reconstruction of the palaeoenvironment can be based on geochemical analysis of samples, concretely carbon. Incorporated into all living organisms, carbon is an essential element. The existence of several carbon isotopes is also well known. Although the factors which cause the incorporation of the heavier isotopes into living organisms are still being researched, some elemental factors are now well known. The isotope ratio can tell us more about the environmental conditions (Farquhar et al., 1989). The positive relationship between the stable isotope values and salinity was proven not only by Ladd and Sachs (2013), but also O'Leary (1981); Farquhar et al. (1982); Werner et al. (2012); Ehleringer et al. (1992); Saurer et al. (2004); Shaheen and Hood-Nowotny (2005). The relationship is best understood as a result of increased water use efficiency at high salinity.

Mass spectrometry is commonly used to measure the isotope ratio. In the localities of the Bohemian Cretaceous basin, the stable carbon isotope ratio has also been analysed by the EA-IRMS method (Nguyen Tu et al., 2002). This method uses all the carbon which is present in the sample and therefore, the results might be not very informative. To overcome this problem, a relatively new direction of geochemical analysis has been developed: Compound Specific Isotope Analysis using GC-IRMS. This method is commonly used for recent materials but it is not often used for analysing palaeontological material.

In this thesis four methods, including the GC-IRMS, are used to specify the environmental conditions at the localities of the Bohemian Cretaceous basin – Pecínov, Hloubětín, Malá Chuchle and Horoušany. Moreover, the disputable species of Ginkgoales from the Saxony Basin in Germany – locality Duingen – is also studied. This locality is also assumed as an environment with slightly salt tolerant flora. To specify the environmental conditions, it is necessary to study a recent ecosystem which is similar to the assumed fossil one, the salt marsh. Samples from recent salt marshes are used as a model environment.

2 Goals

The main goal of this thesis is to establish autecological requirements of selected taxons from the Czech Cretaceous based on carbon isotopes, to understand and describe methods of comparing fossil plants based on macromorphological, micromorphological and chemical characters and to propose a method of reconstructing selected terrestrial environments of the Czech Cretaceous.

3 Theoretical part

3.1 Salt marshes and mangroves

According to the sedimentological interpretation of localities in the Czech Cretaceous (see Section 3.3), similar ecological settings have to be found at present. These ecological settings are the wet coastal ecosystems – maritime salt marshes and mangroves. First, it is necessary to find out if the environment was closer to recent mangroves or more similar to recent salt marshes. For this reason it is essential to know the definitions and basic characteristics of both environments. In both environments the plants had to adapt to growth in saline substrates.

3.1.1 Definition of a salt marsh

A salt marsh is an "area of alluvial or peat deposits, colonized by herbaceous and small shrubby terrestrial vascular plants, almost permanently wet and frequently inundated with saline waters." (Long and Mason, 1983).

We distinguish six different types of salt marshes:

1. **Lagoonal marshes** – they are an enclosed tidal water body with a very narrow connection to the sea. The wave energy is very low as well as the tidal amplitude.
2. **Beach plains** – these are protected by sand or shingle bars but flooded during high tide. They often lie between a salt marsh and a sand dune. The wave energy is low.
3. **Barrier island marshes** – in this case the chain of islands offshore creates a barrier for calmer waters and therefore the salt marsh onshore can evolve.
4. **Estuarine marshes** – there is no physical barrier and as the name suggests – they occur in estuarine environment. The main factor which causes low wave energy is the length and shallowness of estuaries. This type of salt marsh is the most abundant, mainly in middle and high latitudes. Strong tidal currents in contrast to other types are present. Also salinity fluctuates much more thanks to the tidal currents.

5. **Semi-natural marshes** – these marshes have been significantly modified by man, but they still present the remains of a natural salt marsh.
6. **Artificial marshes** – salt marshes created by man. (Long and Mason, 1983)

3.1.2 Zonation of salt marshes

Because most of the marshes are shallow, narrow long areas and the tide is not all the time the same we can distinguish three zones of plants and their associated communities. These zones are based on the height of the tide, see Fig. 1. We distinguish three basic zones: low, middle and upper marsh. The *low marsh* is the lowest part of the marsh, the zone between MHWN (the spring tides, higher than normal one) and MHW (normal tide, mean tide). This part of the marsh is also called the *pioneer zone*. The *middle zone* is defined as the area between MHWN (neap tide, lower than normal one) and MHW (normal tide). The *high marsh* is placed above the MHWS (spring tide) (Long and Mason, 1983). Each of the zones is characterized by the occurrence of certain salt-marsh plant species.

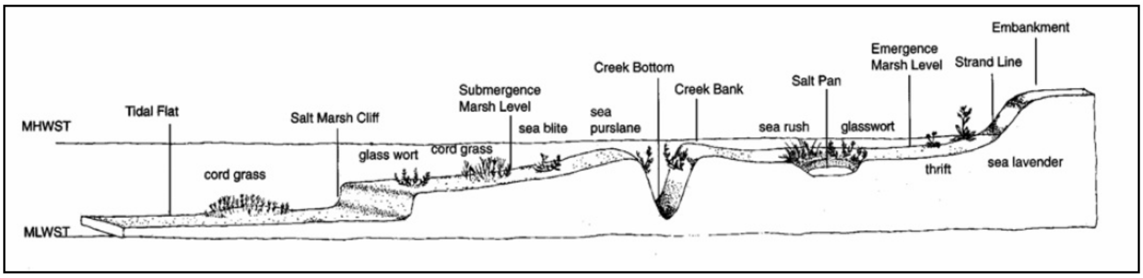


Figure 1: Zones of the salt marshes based on high of tide, (Adnitt et al., 2007).

A different approach to zonation is the *altitudinal zonation* of salt marsh. In some cases this zonation is more useful, because it divides the *vertical range* of the marsh from the seaward limit to the highest point of influence of the tidal waters into three zones: low, middle and high. Despite climatic and geographical differences between various localities, the species living in these three zones follow a similar pattern. The low marsh includes 3–4 species, the middle marsh contains many more species and the major species from the low marsh are usually reduced or absent. The higher marsh is a combination of halophytic and non-halophytic species. This phenomenon is shown in Fig. 2 (Long and Mason, 1983).

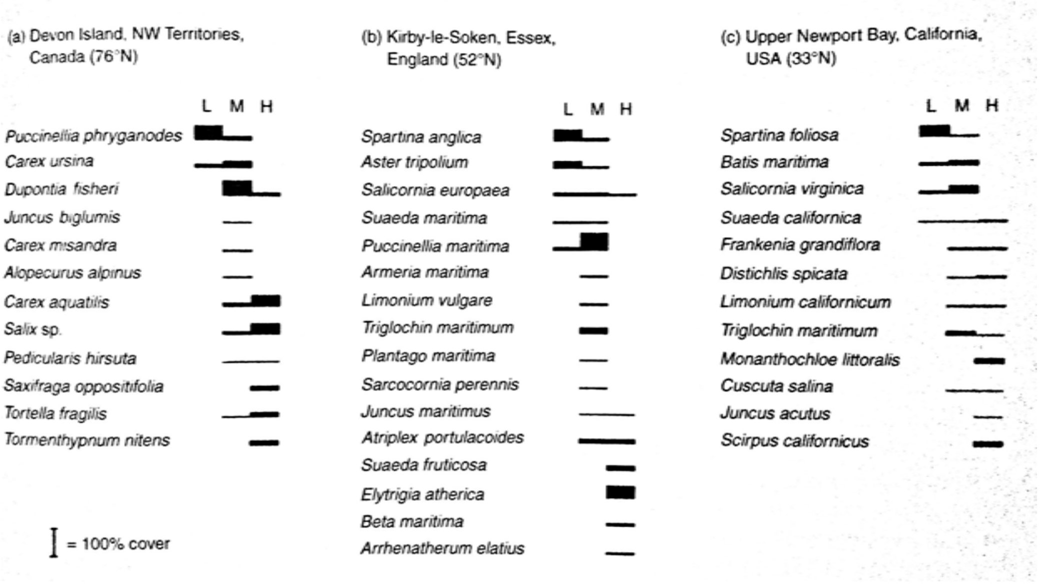


Figure 2: Plant distribution in the three zones of salt marshes, L = low marsh, M = middle marsh and H = high marsh.(Long and Mason, 1983).

The situation in nature is not ideal and therefore the distribution of plants in not just a reflection of altitude within the salt marsh. Thus in a given height spatial variation exists and this may often be related to physiogeographical features. For example in the middle marsh in eastern England there is a dense growth of *Halimione portulacoides* around the creek, but in the same height but dryer conditions there is no occurrence of this species. In the dryer areas there is *Limonium vulgare, Armeria maritinma, Plantago maritima, Puccinela maritima* and *Triglochin maritima.* Othman (1980) observed the distribution of plants in salt marshes by selecting 376 quadrats at random and classified these quadrats using the species composition method introduced by Williams and Lambert (1959). He detected six main groups, but these areas form a mosaic (see Fig. 3). The trend in their distribution is apparent with the distance from the creek. One group is characterized by the presence of *Halimione portulacoides* – near the creek. Away form the creek there are groups characterized by *Halimione portulacoides* or *Plantago maritima.* About 20 m from the creek the quadrates are characterized by *Limonium vulgare, Armeria maritima, Triglochin maritima, Spergularia maritima* and *Plantago maritima.* The most far away form the creek are two groups, one is characterized by *Spartina anglica* and the second by *Salicornia europaea.* For better illustration see Fig. 3.

Soils of the group characterized by *Spartina anglica* have the highest water content for most of the year, but they have the lowest oxygen levels. Soils of the group of *Halimione portulacoides* show the lowest water content, the higher oxygen level and smaller fluctuations of salinity (Othman, 1980).

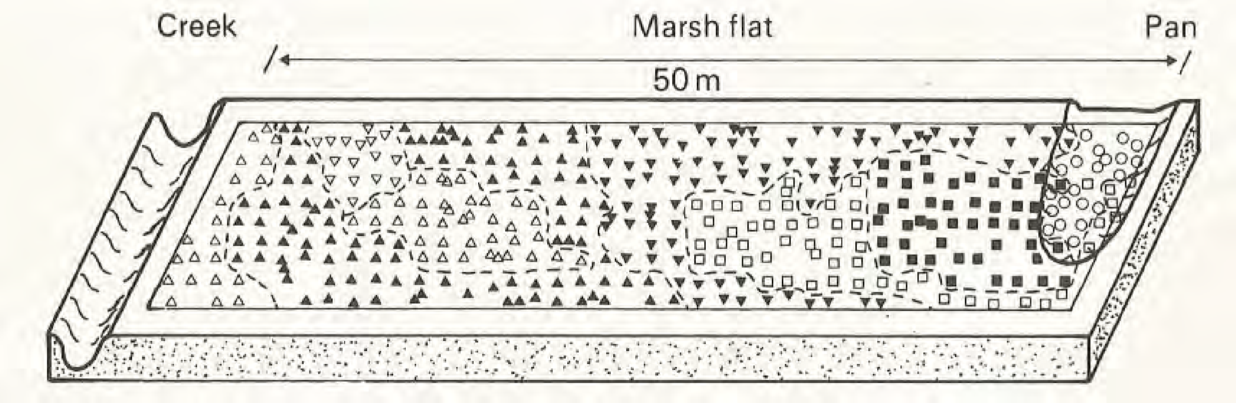


Figure 3: Plant distribution in the mosaic zones of salt marsh, △ – zone of *Puccinela, Halimione*, ▲ – zone of *Puccinela*, ▽ – zone of *Halimione*, ▼ – no dominant occurrence, ■- zone of *Spartina*, □ – zone of *Salicornia* (Othman, 1980).

The zonation is also influenced by the salinity gradient. The salinity of the soil can rise to a much higher level than the salinity level of the sea water because of the evaporation during inter-tidal periods. Therefore different species have a different level of salinity tolerance and this can influence the zonality (Ranwell, 1972). The salt tolerance was observed by Gillham (1957) and the result is shown in Fig. 4.

3.1.3 Definition of a mangrove

Mangroves are defined as environments populated by trees with canopy up to 40 m tall and low scrubby plants (Packham and Willis, 1997). The canopy height depends on climate, topography and the extent of human disturbance (Tomlinson, 1986). Mangroves are situated on tropical coasts where mean temperature doesn't fall below 20°C. Some of the trees, e.g., *Avicennia marina*, tolerate a mean winter temperature of 10°C (Packham and Willis, 1997).

The boundary between salt marshes and mangroves is very thin in the definition and the main factors distinguishing them are the plants and latitude. The absolute limits for mangroves are at 28° N and 25° S–38° S. Basically, where the mangroves end, the salt marshes begin. For a more precise range of occurrence of salt marshes, the northern

Figure 4: Distribution of species in relation to the salt-tolerance. (Gillham, 1957).

limit is 57° N. This limit is controlled, as well as in the case of mangroves' limits, by temperature. In the southern hemisphere the limit is at 35° S and it may be controlled also by day length (Ranwell, 1972). One can see an overlap of mangroves and salt marshes in the southern hemisphere. This is an example of the thin border between the definition of mangroves and salt marshes.

Therefore, what is the difference between the salt marsh and the mangrove? When comparing the salt marsh with mangroves, the main difference is that in mangroves the plants/trees are permanently wet, their roots are under the water level. On the other hand the salt marshes are not permanently wet and the plants have to be adapted for long periods of high evaporation and dryness (Long and Mason, 1983). Another difference is that mangroves are dominated by trees or tall shrubs in comparison with herbal salt marshes (Packham and Willis, 1997).

3.1.4 Mangal characteristics

Mangroves are easily recognized because of their specific plant assemblage consisting of few species. The substrate is firm to soft mud and the most abundant component is the looping aerial roots of *Rhizophora*. The substrate can also be richly organic with peat made up under the mangrove root systems. These muddy environments produce a strong smell of hydrogen sulphide which is an indication the completely anaerobic environment (Tomlinson, 1986).

Also, in the case of mangroves, there is more than one form of mangrove. There are three essential types of mangroves which are based on local factors like shore morphology, the tidal influence and the river flow, variation in salinity and sedimentation.

1. **Riverine mangroves** – mangroves in the river deltas (Indus, Ganga, Amanzon etc.). They are low tidal range, there is strong fresh water flow carrying quite a high amount of sediment which is deposited in mangroves.

2. **Tide-dominated mangroves** – in the tide-dominated estuarine with high tidal range over a shallow inter-tidal zone which can be colonized by mangroves. The mangrove forest usually occurs along the edges of an estuary. Tide can also carry a big amount of material which can be trapped in the mangroves. But also the tidal erosion is very common, therefore the material flux is bidirectional. The influence of wave activity is mainly in the case of the low river discharge, resulting in a steeper shore. Wave-dominated estuaries are unstable and constantly changing in topography. The combination of high river discharge and wave action is perfect for establishing coastal lagoons – habitats for mangroves.

3. **Basin mangroves** – mangroves on the landward side. This environment is out of wave influence, sometimes the high tide carries water and sediment into the basin. Salinity in these mangroves varies very much because of evaporation, which may cause high salinity. On the other hand the influence of ground water flow and rainfall reduces salinity.

These three types of mangroves represent extremes of a continuum, rather than separate forms. It is common that all three types are combined in one delta (Hogarth, 1999).

3.1.5 Zonation of mangroves

Mangrove species zonation can be considered on different scales – vertical sequence of species, regular sequence of species or they can be totally unzoned. An example of vertical sequence (small scale zonation) is the situation on a tide-dominated shore, but the reality is not as simple as can be seen in Fig. 5. Also the vertical sequence can repeat itself on the banks of tidal creeks and rivers (an example is the mangrove in western Malaysia) (Hogarth, 1999).

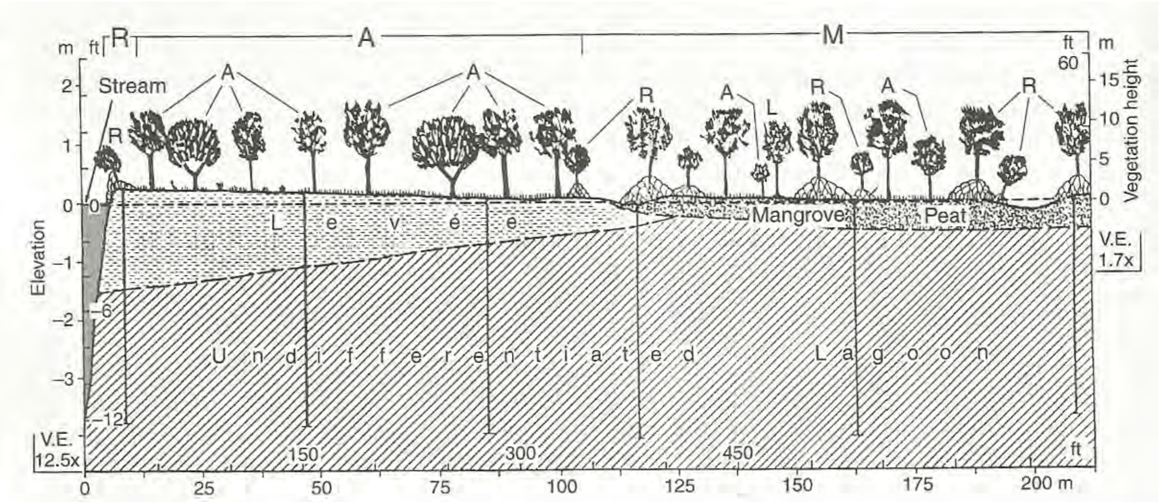


Figure 5: Mangal association – vertical lines represents boreholes, R – *Rhizophora*, A – *Avicennia*, L – *Langularia*, M – Mixed mangrove forest. (Packham and Willis, 1997).

A slightly larger scale is the regular sequence of species zonation and it occurs with increasing distance up the river. On larger scale one can observe regional trends such as the gradation of species across the vast delta of the Ganges and Brahmaputra.

The largest scale of zonation is influenced by geographical trends in the distribution of species. The main factor for global mangrove distribution is the limitation by the temperature – mangroves are exclusively tropical, subtropical in geographical distribution (Hogarth, 1999).

Mangrove zonation in general is caused by four main factors – physical sorting of floating propagules (fruits, seeds), geomorphological changes, ecological interactions between species of the community and also the physical gradients like salinity.

The *propagule sorting factor* was observed in Panama and the result was that the shore level at which a species occurred correlated with propagule size. The high shore species were those with smaller propagules and larger propagules such as *Rhizophora* are established at lower and more frequently flooded levels (Rabinowitz, 1978).

The *factor of geomorphological changes* is mainly caused by erosion and avulsion of the deltaic channels. Also the sea level rise or fall can influence the zonation. It is necessary to be careful in interpreting it, because if the sea level rises or falls rapidly, the zonation may be misleading and may not reflect current conditions. In the case of slow change, mangroves have the time to adjust and the zonation does reflect current conditions. All these geomorphological factors are connected with the physiological factors (Hogarth, 1999).

The *ecological interactions and plant succession factor* is based on the interpretation that the zones represent stages in a successional sequence. From pioneer species which colonize the seaward fringe, the mangrove zonation is continuing into the mature climax forest behind. The extreme form of this theory is that the mangroves are creating land – the pioneer species are trapping mud and consolidating and extending the shore line. Disturbances such as storms are slowing this successional progress (Hogarth, 1999). This theory was discredited by Smith (1992) but parts of this theory, like the trapping of mud, are well known.

The last group of factors are *physical gradients*. One of the factors is the tidal regime which causes gradients in salinity depending on the frequency and duration of submergence. Mangroves might respond to these tidal regimes. The relationship between shore line level and salinity is not straightforward, because of the considerable influence of fresh water seepage or runoff. But essentially, the salinity increases in the seaward direction (Hogarth, 1999). The influence of salinity gradient on the mangal plants has been observed by Ladd and Sachs (2013), and the positive relationship between the stable isotope values and salinity was proven not only by him, but also O'Leary (1981); Farquhar et al. (1982); Werner et al. (2012); Ehleringer et al. (1992); Saurer et al. (2004); Shaheen and Hood-Nowotny (2005). The relationship is best understood as a result of increased water use efficiency at high salinity.

To interpret the zonation of mangroves, it is necessary to keep in mind that the zonation of adult trees may be misleading, because it is just the culmination of all of the relevant processes which have affected all preceding life cycle stages (Hogarth, 1999).

3.2 Stable isotopes as indicators of palaeoenvironment

Because all living plants are gaining energy in the form of hydrocarbon compounds by photosynthesis, the stable isotopes of carbon or hydrogen can be a good indicator of the environmental conditions. Carbon has two stable isotopes: ^{12}C and ^{13}C. During photosynthesis, the lighter isotope ^{12}C is much preferred for biosynthesis to the heavier ^{13}C (Killops et al., 2005a; Farquhar et al., 1989); this is known as the *discrimination phenomenon.*

Because of this discrimination, the heavier carbon is not very abundant in the plants' bodies. Many studies have searched for factors influencing the stable isotope ratio. The main factors described in the literature are water-use efficiency and water availability (Gröcke, 1998). Water availability is connected with salinity in our area of study. Therefore the salinity gradient should influence the vegetation. Then, if we have some zonation in salt marshes or in mangroves, the zonation can be influenced by the salinity, or in other words water availability.

Depending on the metabolism of the plants, there are three groups of plants and each of these groups is adapted for different environments (Nobel, 2009). But the important group for this thesis is the plants of metabolism type C3; this is because the fossil plants in the Cretaceous used this metabolism. The important phenomenon for stable carbon isotope analysis is that the plants are using the heavier isotope for the biosynthesis when water availability is low. Low water availability causes the plant to close stomata and it has to husband with the CO_2 which is enclosed in the plant. When all the light isotope is used, there is no other choice than to use the heavy one. This is how the heavy isotope is incorporated into the plant's body.

Farquhar et al. (1989) established a conceptual model describing an isotopic fractionation during carbon assimilation in C_3 vascular plants. The carbon isotope ratio of C_3 plant tissues is proportional to the ratio of the partial pressure of CO_2 (pCO_2) inside the leaf (c_i) to the pCO_2 in the atmosphere (c_a) according to equation (1),

$$\delta^{13}\mathrm{C}_p = \delta^{13}\mathrm{C}_a - a - (b - a)\frac{\mathrm{C}_p}{\mathrm{C}_a}, \tag{1}$$

where $\delta^{13}C_p$ is the carbon isotope ratio of plant tissue, $\delta^{13}C_a$ is the carbon isotope ratio of the atmosphere, a is the isotopic discrimination due to the diffusion of $^{13}CO_2$ versus $^{12}CO_2$ through air (4.4 ‰), b is the isotopic discrimination imparted during carboxylation by ribulose-1,5-bisphospathe carboxylase-oxygenase (RUBISCO – the primary carbon-fixation enzyme in C_3 plants) ($\sim$ 27‰). C_p/C_a is the ratio of intracellular to atmospheric pCO_2 expressed in parts per million volume (ppmv) (Powers et al., 2008).

According to (Farquhar et al., 1989), the ratio $\delta^{13}C$ is calculated as

$$\delta^{13}\mathrm{C} = \frac{^{13}R_{sample}}{^{13}R_{standard} - 1} \times 1000. \tag{2}$$

Carbon from carbon dioxide generated from fossil belemnite from the Pee Dee Formation (PDB) ($R = 0.01124$) has been set as the reference material for the determination of carbon isotopic ratios (Farquhar et al., 1989). More recently, a new VPDB – Vienna Pee Dee Belemnite standard has been defined by USGS (2016).

Leaf waxes

Waxes are produced by plant metabolism to coat and protect its body in the form of cuticles. Plant waxes are mainly long chain n-alkanes (Killops et al., 2005b). The reconstruction of changes in climate and the carbon isotopic composition of the atmosphere relies on the use of well-chosen biomarkers. Among such land-plant biomarkers, leaf waxes belong to the most widely used (Diefendorf et al., 2011). n-alkanes are the most suitable for the fossil or recent analysis due to their high preservation potential (Cranwell, 1981). No significant carbon isotope fractionation or hydrocarbons has been reported for volatilization, photo-oxidation, thermal-oxidation and microbial degradation processes (Bi et al., 2005). Compound-specific analysis of carbon isotopes is important to understand the response of carbon and water cycles in past climate oscillation. Molecular carbon isotope signatures are less influenced by diagenesis and changes in the sources of organic matter that otherwise complicate interpretation of bulk $\delta^{13}C$ values (Pancost and Boot, 2004).

Long-chain n-alkyl compounds are major components of epicuticular waxes from vascular plant leaves. These compounds are relatively resistant to degradation, which makes them suitable for use as higher plant biomakers. These long-chain compounds (n-alkanes, n-alkanols and n-alkanoic acids) have been identified in recent and also in fossil terrestrial sediments and macroremains. *n*-Alkanes are present in extracts of vascular plant leaves and the typical chain-length is ranging from C_{25} to C_{35} (Pancost and Boot, 2004). There is a strong predominance of odd-carbon-number homologues over even-numbered ones, the most common being C_{27}, C_{29}, C_{31} and C_{33}. The stability of these long chains is rising with their length (Cranwell, 1981). Leaf wax composition and abundance can vary with ontogeny, environmental variables, stresses, plant families (Diefendorf et al., 2011) and also within year (months and seasons) (Eley et al., 2016).

Carbon isotope fractionation between atmospheric CO_2 and leaf carbon is well characterized for different photosynthetic pathways (C3, C4 and CAM) and it is also correlated with different plant functional types (angiosperms, gymnosperms, deciduous and evergreen) (Farquhar et al., 1989). The fractionation is influenced by environmental factors, particularly by water availability. These relationship can thus be used for prediction of carbon isotope fractionation and δ^{13}C values of biomarkers in the geologic past (Diefendorf et al., 2010).

3.3 Geological settings and sedimentology

The Peruc-Korycany Formation of the Bohemian Cretaceous basin offers a number of fossiliferous localities (see Fig. 6), e.g., Pecínov, Horoušany, Hloubětín, that yielded very rich palaeofloras. This Formation is formed by fluvial, paralic Peruc Member, shallow marine clastic deposits of Korycany Member and offshore shales to marls of Pecínov Member (Uličný et al., 1997a), see Fig. 7.

3.3.1 Pecínov

The material studied in this thesis comes mainly from the Pecínov quarry locality, which has been observed for more than 20 years. The fossil samples have been collected from Units 3 and 5 of this quarry and also from other localities from Czech Cretaceous (see Fig. 7). The Pecínov quarry is located 40 km to the west of Prague, near the village of Rynholec.

The Cenomanian deposits of the Bohemian Cretaceous Basin include one of the richest and best preserved fossil floras in Europe, especially the Peruc-Korycany Formation. The studied environment was formed during the stepwise transition from fluvial

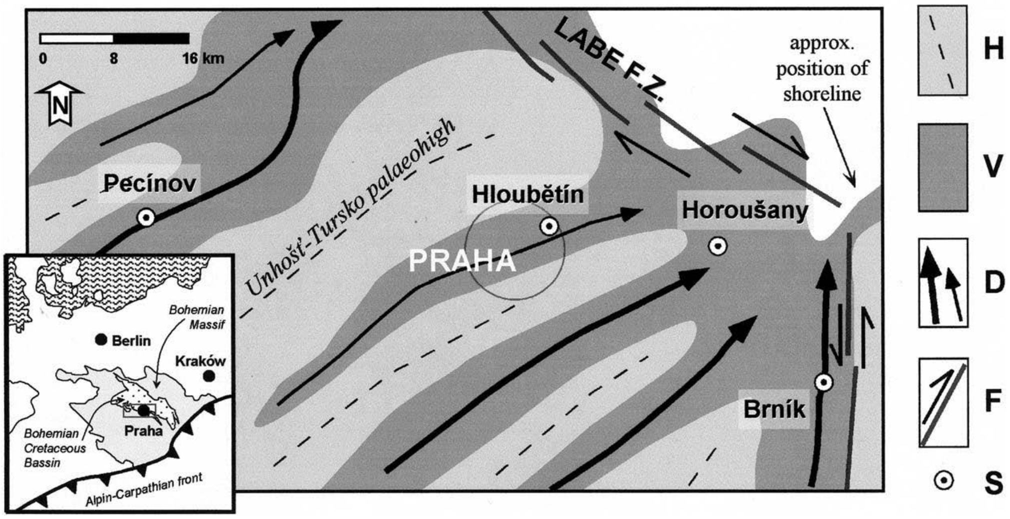


Figure 6: Schematic palaeogeographic map of study area during the early late Cenomanian. F.Z. – Fault zone, H – palaeohigh and palaeohigh axis, V – palaeovalleys and coastal plains, D – valley axis and direction of stream systems, F – major fault zone/sense of movement, S – sampling sites (Nguyen Tu et al., 2002).

to tide-dominated, estuarine sedimentary facies (Uličný et al., 1997b). During the mid-Cretaceous, west-central Bohemia was situated at the south-western margin of the Bohemian Cretaceous Basin. This margin had a low subsidence rate and was generally flat. There was low sediment input from the flat drainage area of the south-western part of the Bohemia Massif (Uličný et al., 1996). The basin was formed by reactivation of a fault system in the Variscian basement of the Bohemian Massif. The reactivation was caused by intra-plate stresses transmitted from the Alpine-Carpathian orogenic front (Uličný et al., 1997b). The initial late Cretaceous transgression during the late Middle Cenomanian created an estuarine depositional setting by flooding a system of broad, north-eastern-directed palaeovalleys which were separated by flat palaeohighs (Fig. 8) (Uličný et al., 1996). Because of low subsidence rate, the most important control on the relative sea-level change in this part of basin was eustasy.

The collected samples are from the Peruc-Korycany Formation which is divided into five units (Fig. 9) based on the sedimentary textures. These members were formed in Cenomanian by fluvial, marginal marine (Peruc Member), shallow marine (Korycany Member) clastic deposits and offshore shales to marls (Pecínov Member). The Peruc-Korycany Formation is the oldest part of the Bohemian Cretaceous basin fill and reflects the initial transgression during the Cenomanian. During the late middle Cenomanian a

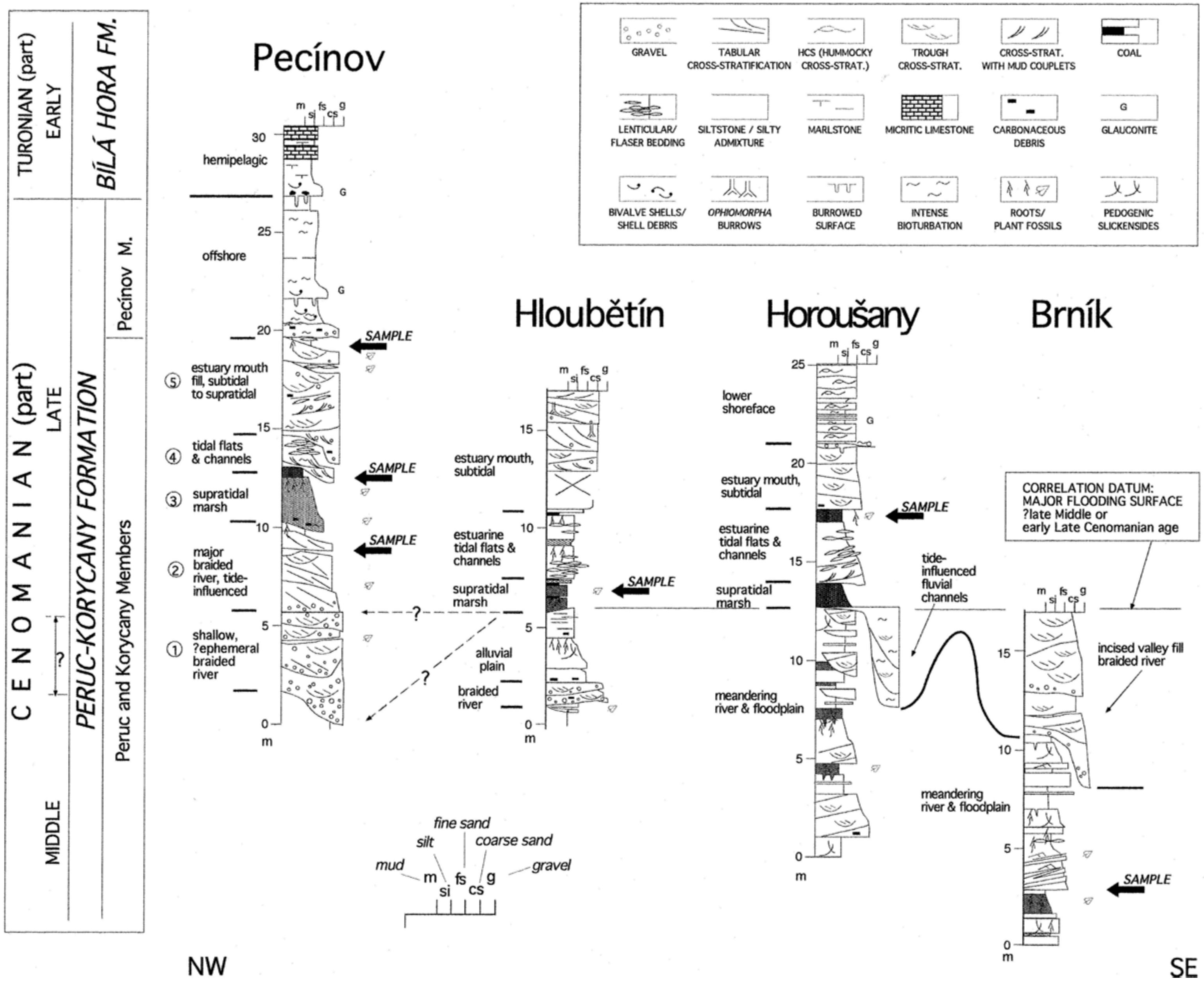


Figure 7: Correlation of three localities of Peruc-Korycany formation (Nguyen Tu et al., 2002).

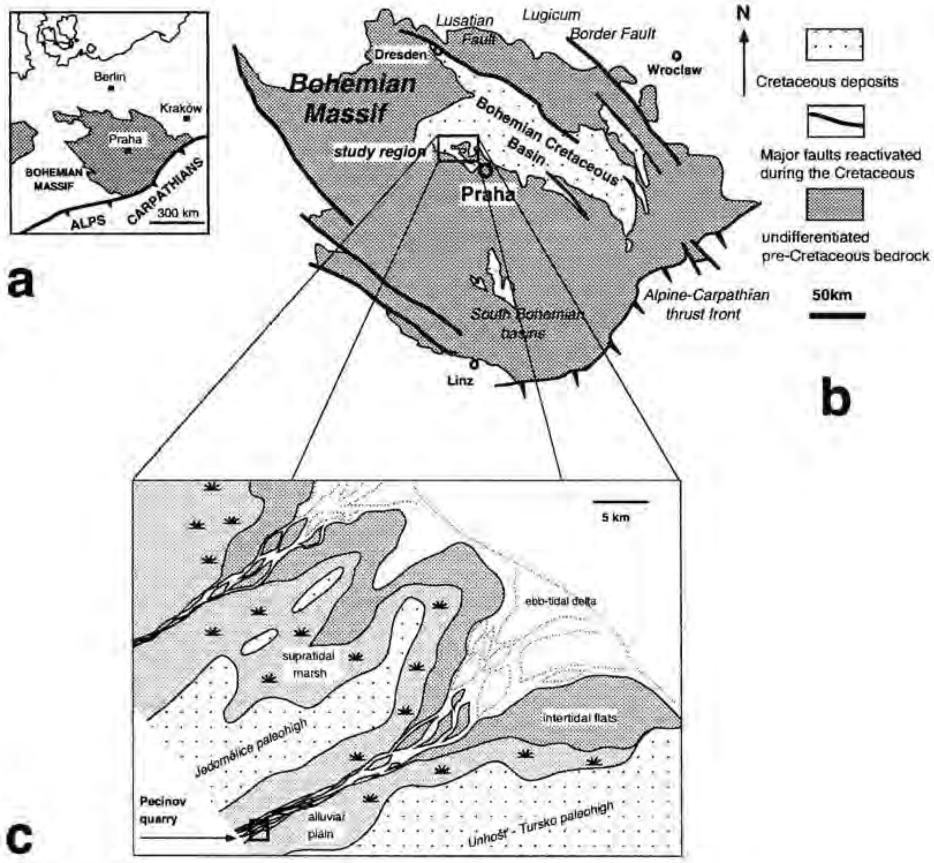


Figure 8: (a) Position of the Bohemian massif in Central Europe. (b) The Bohemian Cretaceous basin. (c) Palaeogeographic reconstruction of the surroundings of the Pecínov quarry (during deposition of unit 2 at Pecínov), (Uličný et al., 1997b).

rapid sea-level rise caused flooding of most of the basin. After another major sea-level rise, on top of the Korycany Member, dark offshore shales of the Pecínov Member were deposited (Uličný et al., 1996).

The interpretation of the five depositional units is based on the sediments and flora macroremains. Units 1–2 were alluvial environments in the proximal to medial part of palaeovalley dominated by angiosperms gallery forest with conifers and cycadophytes becoming increasingly abundant towards the coast – Units 3–5. Retrograding coastal environments were dominated by halophytic cheirolepid, taxodioid or ginkgo-conifer forests and shurb – Units 3–4. The prograding coastal environments were dominated by mesic taxodioid conifer, ferns forests – Unit 5 (see Fig. 8) (Falcon-Lang et al., 2001).

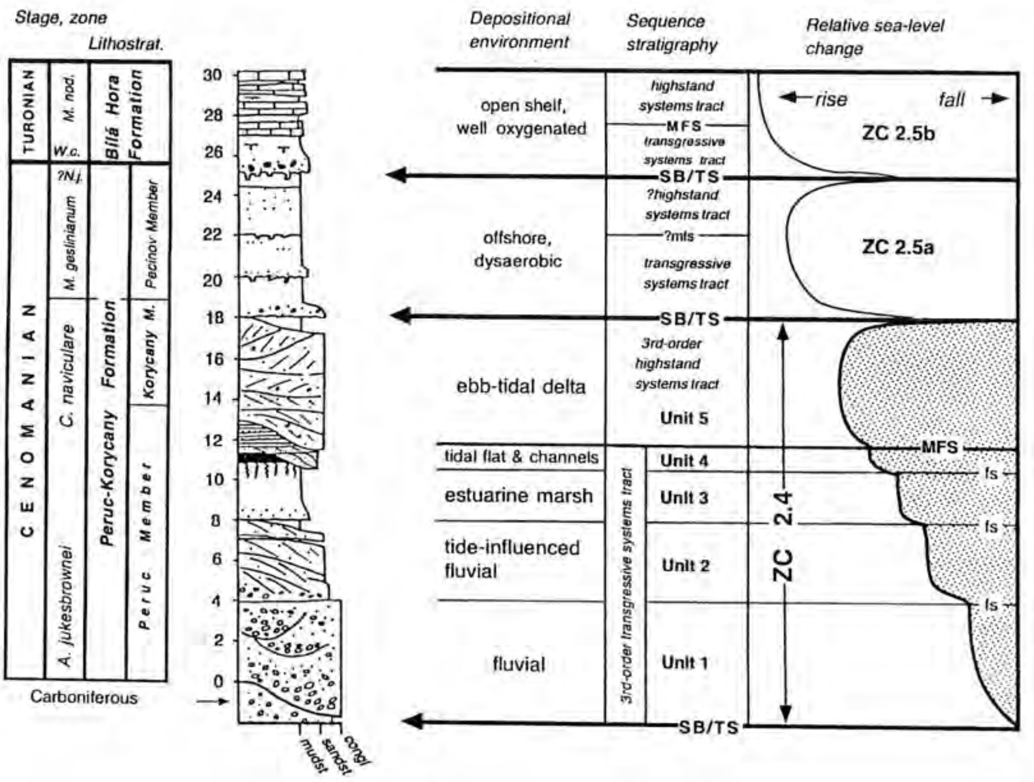


Figure 9: Stratigraphy and depositional environments of Pecínov section (Uličný et al., 1996). Units 1–5 (parasequences) are the fluvial to estuarine deposits of the Peruc and Korycany members. It indicates the sea-level fluctuations. *SB=sequence boundary, TS=transgressive surface, fs=flooding surface, MFS=maximum flooding surface* (Uličný et al., 1997b).

Unit 1: Gravelly braided river This unit has up to 6 m of thickness and rests unconformably on Carboniferous conglomerates and arkoses. It is absent on local palaeotopographic highs and the basal surface is erosional. Channels are 1–10 m wide and 0.5–1 m deep, cross-cutting one another vertically as well as laterally. The channel fills are poorly sorted conglomerates with cobbles, coarse to medium grained sandstones and mudstones. This unit is rich in angiosperm leaves – *Platanus laevis*, *Myrtophyllum geinitziia*, *Grevilleophyllum constans* and includes accesoric elements of *Nilsonia holyi* and *Antocephale chuchlensis*. The palaeoenvironmental interpretation of Unit 1 is that it represents deposits of shallow, gravelly braided river which began to accumulate due to a gradual ride in sea level, which caused a landward shift of the shoreline and the backfilling of the valley. The channel migration was quick and frequent due to fluctuations in discharge. The plant fossils are preserved in remnants of muddy infills of temporarily abandoned channels (Uličný et al., 1997b).

Unit 2: Tide-influenced braided river Unit 2 can be up to 5 m thick and rests on the almost flat surface of Unit 1. Locally it can rest on Carboniferous bedrock – in the areas where Unit 1 is absent, mostly filling the topographic lows. As the dominant lithology of Unit 2 there are fine-grained conglomerates and pebbly sandstones. Also fine sandstones, planar cross-bedded or though cross-bedded sandstones with abundant mud drapes on foresets are present. The fossil plants occur in dark, sandy mudstones interbeds in the heterolithic sandstone facies. Very common plant macrofossils are *Myrtophyllum geinitziia* and *Grevilleophyllum constans*. The sediments of Unit 2 represent a large, low-sinuosity (braided) river with tidal influence. Because of the rising sea level this palaeovalley was slowly flooded. The alluvial plain was present and the trees, shrubs and herbs grew in more humid conditions then in Unit 1 (Uličný et al., 1997b).

Unit 3: Supratidal marsh This unit is very important, because the studied fossil samples are directly from this supratidal marsh unit. It rests sharply on the fluvial sediments of Unit 2. Locally we can find incision of the channels from Unit 4, which reduced the normal thickness of Unit 2 from 3–3.5 m to less than 1 m. The main lithology of this unit is created by dark grey to black mudstones, rich in pyrite concretions. Also the carbonaceous mudstone is present. At the base of the unit, the grey sandstones with muddy interbeds usually occur. The channel-fill bodies are up to 30 m wide and 4 m deep, filled by lateral accretion of sandstone-dominated heterolithic lithofacies. Also dark silty rhythmic lamination can be found in the channel fills. In this unit there are two different assemblages of fossil plants (Fig. 10):

1. In grey to dark-grey mudstones channel-fills: there is abundant phytodebris and fragmented plant remains. The main taxa are *Frenelospis alata*, taxodiacous conifers *Sequoia major* and other xerophytic like taxa. The sedimentology and palynology indicate that this area was influenced by brackish or marine water.

2. In peat-like sediment at the top of Unit 3: this assemblage has abundant leaves compressed together in large quantities. The main taxa are *Frenelospis alata* and *Nehvizdyella obtusa*. This assemblage is similar with locality Hloubětín which represents supratidal facies. The presence of *Frenelospis alata* suggest marine water influence. The palaeoenvironmental situation indicates that the marsh deposits sed-

imented on flooding surface created by sea-level rise which shifted the originally fluvial environment into the intertidal and supratidal conditions (Uličný et al., 1997b; Fatka and Kvaček, 2006).

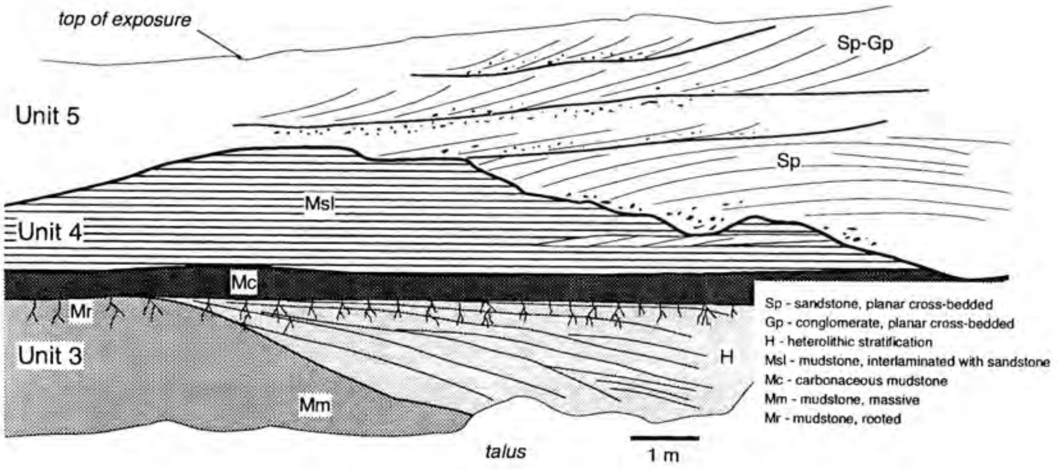


Figure 10: Marsh mudstones and tidal-creek channel fill sediments of Unit 3, overlain by intertidal laminites of Unit 4 and subtidal sandstone of Unit 5. The rootlet zone below the carbonaceous mudstone (Mc) is capping Unit 3 (Uličný et al., 1997b).

The plant fossils in the channel-fills of Unit 3 represent the partly transported vegetation of backswamps (situated more inland – taxodiacous conifers) and also the local, autochtonous flora (*Frenelospis alata*). The second assemblage represents halophytic vegetation dominated by *Frenelospis alata* and *Nehvizdyella obtusa*, which grew at the level of upper tidal limit on the tidal creek margins of salt marsh. Because of the shrubby character of the vegetation there is suggestion of “mangrove-like” community. But not only shrubby vegetation was probably present, also herbaceous pteridophytes, angiosperms (*Pseudoasterophyllites*) (Kvaček, 2016) and bryophytes are suggested by the pollen spectra (Uličný et al., 1997b).

Unit 4: Tidal flat and channels Unit 4 has sharp erosional boundary with Unit 3 with locally deep erosional channels (up to 3 m) into Unit 3. Because of the erosion by Unit 5, the sedimentary record of Unit 4 is not very large. The lithology of channel-fill bodies is planar cross-bedded, coarse to medium-grained sandstone with mud-draped presets and mud couplets. This lithology indicate an asymmetrical tidal current regime in a subtidal environment. The classical tidal bundles organized in bundle sequences reflecting neap-spring tide cyclicity can be found. There are no plant macrofossils from this unit. Sediments of this unit represent a mixed sand and mud tidal flat. The main reason for the absence of plant macrofossils is the strong influence of tidal currents. The tidal flat was probably not vegetated (Uličný et al., 1997b).

Unit 5: Ebb delta to estuary mouth infill Samples from the highest unit, Unit 5, were also used in this thesis. This unit is up to 11 m thick and shows a distinct shallowing-upward pattern – vertical transition from subtidal to supratidal facies. The erosional boundary can also intrude around 5 m into the underlying Units 4 and 3. The Pecínov member is loaded on this unit. The main lithology is coarse-grained sandstones with planar and trough cross-bedding (Fig. 10). Near the channel bottoms the mudstone rip-up clasts or fustian clasts can be found. The sedimentary geometry is different in each part of the channels – the lower part has lateral infills, whereas the middle part has lateral-accretion sandstone lithosomes. The direction of palaeocurrent indicates dominant basinward (ebb) directions. The upper part of this unit has heterolithic lithologies and mudstone channel fills. These mudstone channel fills are dark and organic rich with faint lamination and lenticular bedding. The mudstones are rich in macrofloral remains and the top of Unit 5 is densely penetrated by coalified rootlets. The silicified pseudotrunk of the fern *Tempskya varians* is present in sandstones and conglomerates. The channel-fill mudstones contain fossil plants characterized by lack of angiosperms. The most common taxa are taxodiaceous conifers *Sphenolepis pecinovensis* and *Cunninghamites lignitum*. Whole twigs sometimes with cones attached can be found in this unit. The fossil plants have mesophytic character and are generally allochthonous. Plant remains were transported only a short distance, therefore they are quite well preserved. Plants were not showing xerophytic micro-morphologies like in Unit 3. Mainly the ferns and lycopods were growing on the marsh. Sediments of Unit 5 are interpreted as progradational tide-dominated coastal succession. It is shallowing upward from subtidal estuarine ebb-tidal deltaic deposits to muddy and heterolithic intertidal deposits filling the channels on prograding coastal plain formed after filling of the estuary mouth. The sandstone facies reflect the slope of the ebb delta lobe and also show the lateral accretion within shallow channels and sandstone sheet bodies. The coarse grain size of sandstones indicates deposition relatively near the estuarine mouth. Muddy and heterolithic facies of intertidal mudflat and supratidal marsh overlay on the sandstones and conglomerates (Uličný et al., 1997b).

3.3.2 Horoušany and Hloubětín

The sediments of Horoušany quarry come from the deepest palaeovalleys filled in Cenomanian. The age of fluvial deposits of the Horoušany section was determinated from the palynologycal spectra to middle Cenomanian. The overlying tide-dominated estuarine and storm-dominated shore-face deposits are of late middle to late Cenomanian age.

The sediments in locality Hloubětín contain only a thin portion of fluvial origin at the base and the upper part of Hloubětín is mostly analogous to the upper part of Horoušany. Comparing with locality Pecínov (see Fig. 7), the fluvial deposits in Pecínov are approximately time-equivalent to salt marsh or intertidal estuarine deposits in Horoušany and Hloubětín. Therefore the Pecínov locality was a more landward part of the preserved basin fill (Nguyen Tu et al., 2002). Figure 11 shows a model of palaeoenvironmetal conditions of Units 1–5 of the Peruc-Korycany Formation and illustrates the palaeoenvironments of above described units.

3.3.3 Duingen

The material from the locality Duingen comes from the former clay pit Grube Block, about 1.5 km west-southwest of Duingen, Bückeberg Formation of Lower Saxony in Germany (see Map 12). Duingen is a terrestrial Early Cretaceous deposit of Wealden facies of Western and Central Europe. These facies were deposited under freshwater conditions with episodic marine ingressions in a humid, subtropical climate. The lower part was deposited in brackish-limnic conditions and the upper part has marine settings. The grey clay stones are alternated with dark grey clay stones, and sporadically we can find thin carbonate concretions. Samples from this locality are of the Berriasian age (the lowest Early Cretaceous) (Pott et al., 2014).

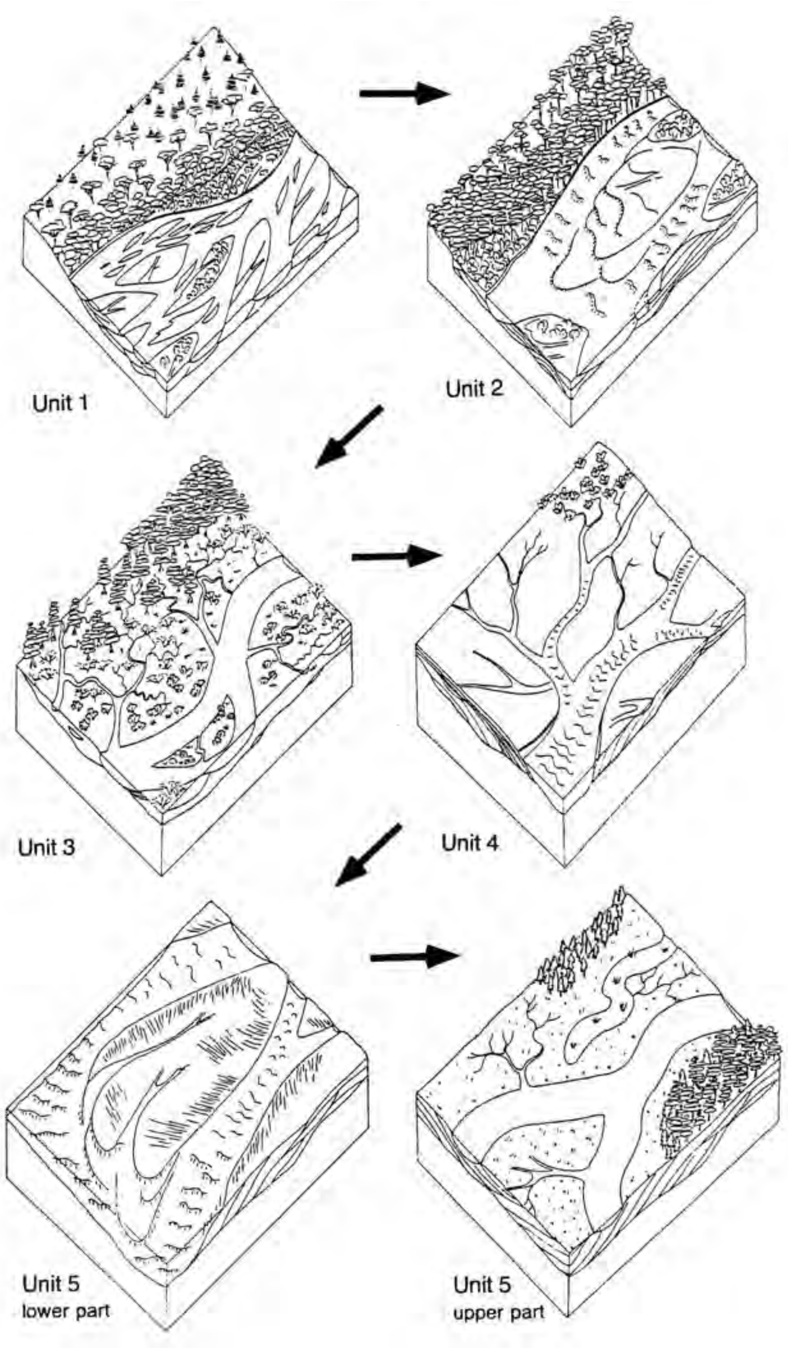


Figure 11: Palaeoenvironmental conditions of Units 1–5 of the Peruc and Korycany Members (Uličný et al., 1997b).

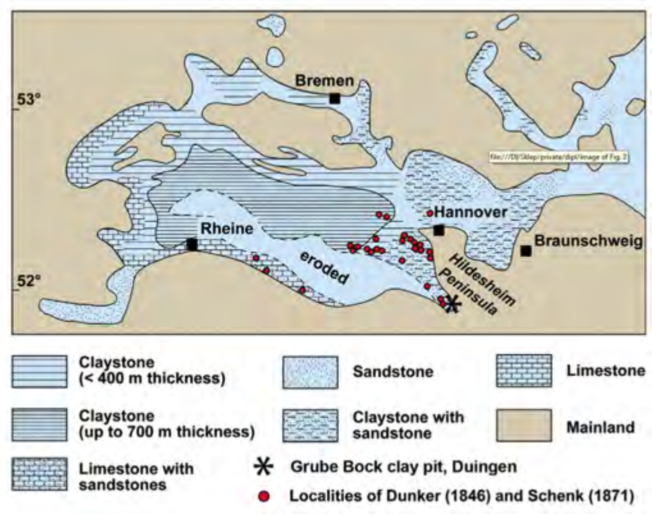


Figure 12: Palaeogeography map of the Northern German Wealden Basin with fossil localities of Duingen (Pott et al., 2014).

4 Material and Methods

4.1 Fossil material and localities

Fossil material was chosen from different localities to cover the whole stratigraphy range of the Cretaceous (also Jurassic) and to obtain a wider regional image of the palaeoenvironment. The localities from the Bohemian Cretaceous basin are Pecínov, Horoušany, Hloubětín and Malá Chuchle. More samples were obtained from the Great Britain Jurassic of Yorkshire – Scalby Mills. We used also samples from the German Lower Cretaceous – Duingen.

From most of these localities, I sampled both assumed halophytes or salt tolerant plants as well as some non-halophytic plants in order to enable the comparison between the two. Another reason for choosing these samples was to prove the autecology of each species. As a reference of halophytic plants we used *Frenelopsis, Eretmophyllum, Pseudoasterophyllites, Tritaenia.* As a reference of the fresh water non-halophytic plants we used lauroid angiosperms and *Ginkgo huttonii* from the Jurassic of Yorkshire, GB (see Plate 1).

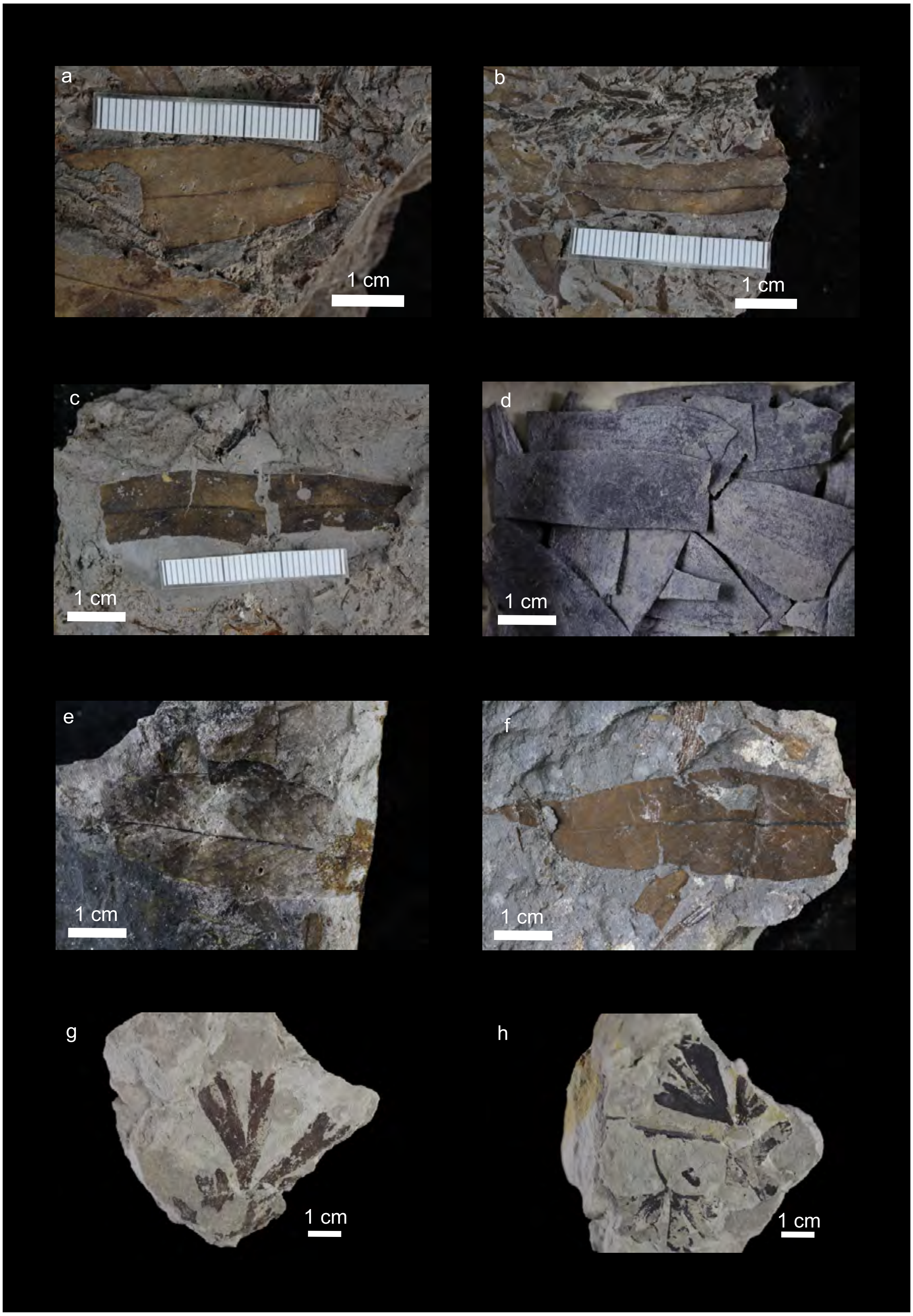


Plate 1: Marco-photos of the fossil samples: (a)–(c) lauroid angiosperms from Pecínov, (d) *Eretmophyllum* from Hloubětín, (e) and (f) lauroid angiosperms from Horoušany, (g) and (h) *Ginkgo huttonii* from Scalby Mills, Yorkshire, GB.

Figure 13: Salt marsh in Little Neston – 53°16′23″N, 3°03′57″W (Google, 2016).

4.2 Recent material and localities

The recent plants were collected to model an ecosystem similar to the fossil one. Based on sedimentological data, the environment was interpreted as a salt marsh ecosystem with trees, shrubs and herbs. In the fossil record there are usually trees and shrubs; herbs are only rarely preserved (e.g., *Pseudoasterophyllites*). Therefore the goal was to find equivalent plants or at least plants which could be used for the stable isotope analysis. The halophytic plants were collected from three salt marshes in Great Britain: Little Neston (53°16′23″N, 3°03′57″W), King's Lynn (52°58′21″N, 0°38′10″E) and Grange-over-Sands (54°09′20″N, 2°56′24″W), 5 or 6 species from each collecting point (Fig. 13, 14, 15).

Another goal was to find some gradient in the salt marsh based on changes of salinity and because most of the fossil samples are remains or parts of trees, it was necessary to find recent equivalents. The absence of trees at recent salt marshes led to the use of samples from recent mangroves – *Rhizophora* (samples from collection of the National Museum, Prague, the Everglades, J. Kvaček, 2006).

Not all species were present at the same place; Fig. 16 shows which species were collected in which place. This diagram shows all the three zones of a salt marsh – low, middle and high marsh – which are based on the vegetation composition (see Chapter 3.1.2).

Figure 14: Salt marsh in King's Lynn – 52°58′21″N, 0°38′10″E (Google, 2016).

Figure 15: Salt marsh in Grange-over-Sands – 54°09′20″N, 2°56′24″W (Google, 2016).

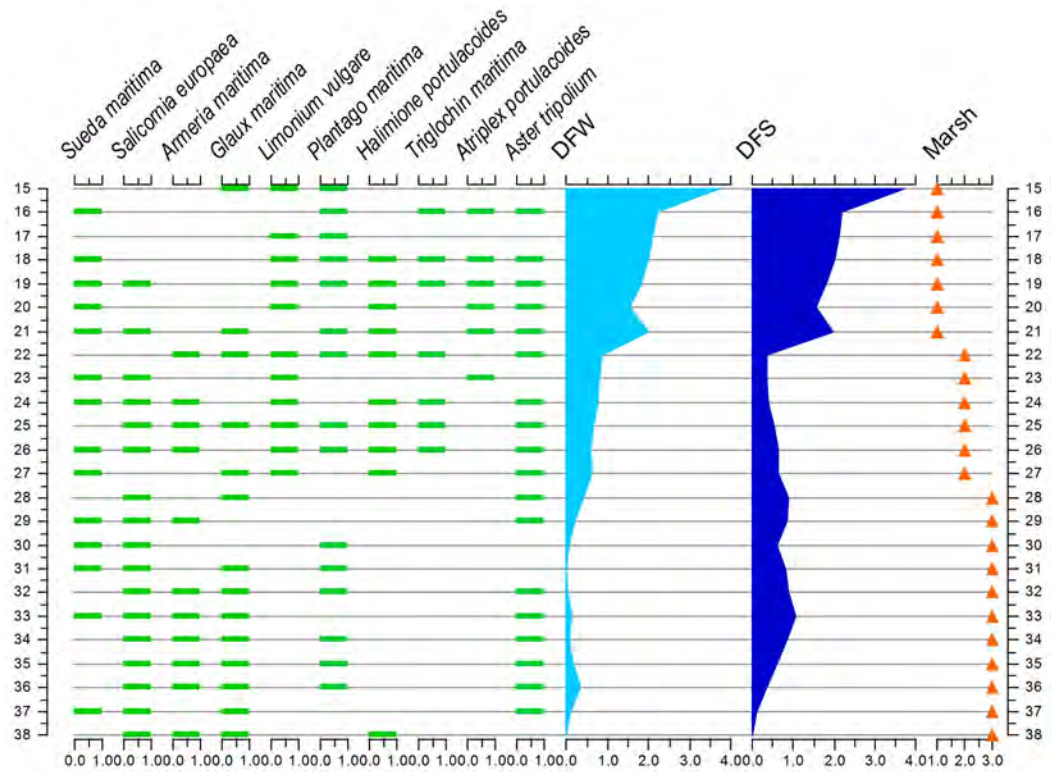


Figure 16: Species distribution of all three marshes. DFS = distance from sea, DFW = distance from water (creek), marsh = 1 – Little Neston – high-middle marsh, marsh = 2 – King's Lynn – middle-low marsh, marsh = 3 – Grange-over-Sands – low marsh. The numbers on the y-axes are used (together with a species identifier) to identify the sample (e.g., SUE16); these identifiers are used in data processing and in plotting the results. Created in C2 (Juggins, 2016)

4.3 Cuticle analysis

Cuticle analysis is a very powerful tool in palaeobotany. Thanks to the cutinised layer covering the epidermis, the look of epidermal cells is preserved even though the material is from Cretaceous. Most of the organic matter is changed into coal or totally degraded, but the cuticle, built of very stable wax components – cutan and cutin – remains.

For cuticle analysis, it is necessary to separate a part of leaf lamina including cuticle and coal material from the rest of the plant fossil. I used sampling by preparation needle followed by HF, Schülze solution and KOH. HF (40%) were used for cleaning the samples for 5 to 10 minutes. After cleaning, the samples were macerated in Schülze solution ($KClO_3$ with HNO_3) for 10 minutes to 12 hours (depending on the material, until the sample wasn't brownish-yellow coloured). To finish the maceration it was necessary to use KOH (concentration 10%) for 5 minutes to a few hours (until the sample wasn't transparently yellowish). Between all these procedures the samples were flushed by water 5 to 10 times. Samples were stored in glycerine or directly prepared for SEM. Samples were observed under the light microscope Olympus BX40, Olympus SZX12 and under the SEM Hitachi S-3700N in the National Museum in Prague.

4.4 Stable isotope analysis

The samples of Cenomanian Peruc Flora are considerably interesting because all the plants probably used the C3 photosynthetic pathway and also the fossil-rich sites occupy a relatively small region in a relatively narrow stratigraphic interval of the middle–early late Cenomanian. The global environmental parameters such as the mean isotope composition of atmospheric CO_2 and temperature did not vary significantly among the sections studied. Therefore the stable carbon isotope ratios of the Bohemian plants were primarily controlled by local environmental conditions such as soil-water content, soil-water salinity or irradiation.

The stable carbon analysis is one of the proxies which tell more about the palaeoenvironmental conditions (Nguyen Tu et al., 2002). Because of the difference between Bulk stable carbon analysis and Compound Specific Isotope Analysis (Eley et al., 2016), both of these methods were applied to allow us to compare results and to investigate which of these methods is more suitable for reconstructing fossil palaeoenvironments. Both of these methods are processed by IRMS – isotope ratio mass spectrometry.

4.4.1 EA-IRMS for Bulk C

After collecting recent samples of plants, the plants were dried and stored in paper envelopes. The stable carbon analysis needs just a small amount of every sample (plant); the sample is packed into special aluminium cups for EA-IRMS. The elemental analysis was measured with an NC 2500 connected to a Thermo Quest Delta+XL mass spectrometer in a stable isotope laboratory in Stuttgart. Samples were calibrated to δ^{13}C values of USGS 24 ($\delta^{13}C = -16.00‰$, relative to VPDB). The reproducibility is ±0.1‰ for δ^{13}C measurements. The reproducibility for carbon content is ±10%. The results are data of bulk δ^{13}C. The total number of measured samples was 105.

4.4.2 Extraction of leaf waxes – preparing samples for GC-IRMS

The first step in the preparation samples for GC-IRMS is the extraction of leaf waxes from samples. Leaf waxes *n*-alkanes were prepared following the method of Eley et al. (2012, 2014); Pedentchouk (2014). The samples were sonicated in hexane (VWR, used for all analysis) (2 × 30 minutes, the fossil samples 30–45 minutes) to obtain total lipid extract. The extract was collected in 40 ml ASE vials (Labicom) and therefore it was concentrated by evaporation in a thermoblock to the volume of 1 ml. *n*-Alkanes were separated from the total lipid extract by column chromatography. A glass Pasteur pipette (150 mm long) was packed with silica gel (high-purity grade (Davisil Grade 635); pore size 60 Å; 60–100 mesh, 150–250 µm; Sigma-Aldrich) and the total lipid extract was loaded onto the column. The column was eluted, before and after loading the extract, with hexane (5–6 ml) and the final eluent containing *n*-alkanes was collected in 8 ml-glass vials (Labicom). For column chromatography we used an SPE Manifold (Waters) with 12 positions. One chromatography column was used for each sample. Eluents were concentrated under the stream of high grade nitrogen (Nitrogen purity 4, Linde) and heated by thermoblock at the same time. They were totally blown down and then dissolved in 100 µl of hexane. The fossil samples were washed by 300 µl and blown down again under the nitrogen in the thermoblock for the final volume of 100 µl. The final volume was injected into 1.8 ml HPLC vials (Labicom) with 350 µl inserts (Labicom) inside. Samples were stored in a fridge.

4.4.3 GC-IRMS, analysis of n-alkanes

Before measuring it was necessary to carry out some basic maintenance like the linearity test and on/off test. In addition, it was required to test if all the parameters are correct – CO_2 gas tank values. Then the Delta V Advantage ThermoFisher IRMS was set for the method for measuring leaf waxes. The carbon isotope composition of *n*-alkanes was determinated using a Delta V Advantage ThermoFisher isotope-ratio mass spectrometer interfaced by CG-Isolink II Trace GC combustion and high temperature conversion systems, using the capillary column (Restek DB - 5MS, $30\,\mathrm{m} \times 0,25\,\mathrm{mm} \times 0,25\mu\mathrm{m}$). The oven temperature was raised from 50 ℃ to 210 ℃ at 15 $℃\,\mathrm{min}^{-1}$, then at 7 $℃\,\mathrm{min}^{-1}$ to 320 ℃. *n*-Alkanes were identified by comparison of their elution times with n-C_{16} to n-C_{30} alkane standard (A. Schimmelmann, Indiana University). δ^{13}C values of *n*-alkanes are reported based on duplicate (in some cases triplicate) analysis of well-resolved peaks and expressed relative to Vienna Pee Dee Belemnite (VPDB) based in in-house reference gasses CO_2 adjusted daily using a standard mixture of n-C_{16} to n-C_{30} alkanes (A. Schimmelmann, Biogeochemical Laboratories, Indiana University). Reproducibility of reference gas peak CO_2 δ^{13}C values (analysed at the beginning and end of each sample measurement) did not exceed $\pm 0.063‰$ ($n = 183$). The total number of measured samples was 183.

4.5 Processing data

4.5.1 Data from EA-IRMS

The data from the EA-IRMS analysis were used for calculation of mean Bulk C values. The samples were sampled in three places on the leaf, mainly on the base, in the central part and in the apex. Because the values of δ^{13}C are fluctuating within the leaf without any pattern of distribution (see Fig. 17), the average value was calculated. The average was used and dispersion calculated between duplicate measurements, for this processing MS Excel 365 was used. Data were sorted and plotted in R!.

4.5.2 Data from GC-IRMS

The data from the GC-IRMS analysis were processed in ThermoScientific Workspace software, MS Excel and plotted in R!. The first step was to identify *n*-alkanes peaks by comparing with measured n-C_{16} to n-C_{30} alkanes standard (A. Schimmelmann, Biogeochemical Laboratories, Indiana University). For determination of the peaks, an important factor was the retention time of the main peaks. The most common alkanes were n-C_{25}, n-C_{27}, n-C_{29} and n-C_{23}. Some of the samples had also high peak in retention time of n-C_{31} or n-C_{33}, but these alkanes could not be used for comparing with the fossil samples. After the detection of *n*-alkanes, the average of each duplicate or triplicate was calculated. The recent samples were sorted by the marsh and all the samples were sorted by the *n*-alkanes. The data were plotted to find a gradient for each species and to find relative relationship between the samples.

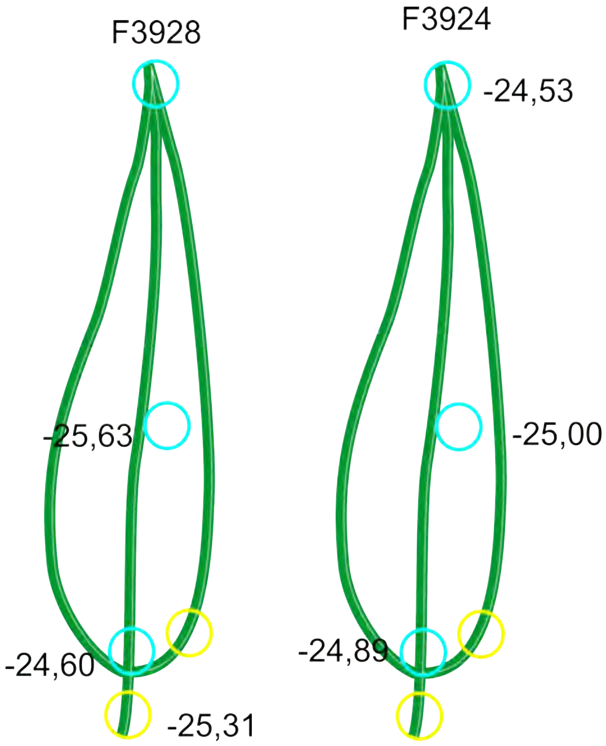


Figure 17: Two examples of sampled leaves, the values are not creating any gradient within the leaf, the average value was used.

4.5.3 Calculating model $\Delta_{LEAF-MAP}$ and $\Delta_{LEAF-LIPID}$ from the measured data

Because the fossil data cannot be compared with recent data, mainly because of the climate change from the Cretaceous until today, it is necessary to edit data into a form which can be compared. The core of this method is to compare edited measured data with modelled data based on MAP (mean annual precipitation). The model data are constructed and calculated based on MAP because the main influence on the δ^{13}C composition is water availability and it is connected with precipitation. The calculations followed the method of Diefendorf et al. (2015) and Arens et al. (2000). The idea is shown in Fig. 18

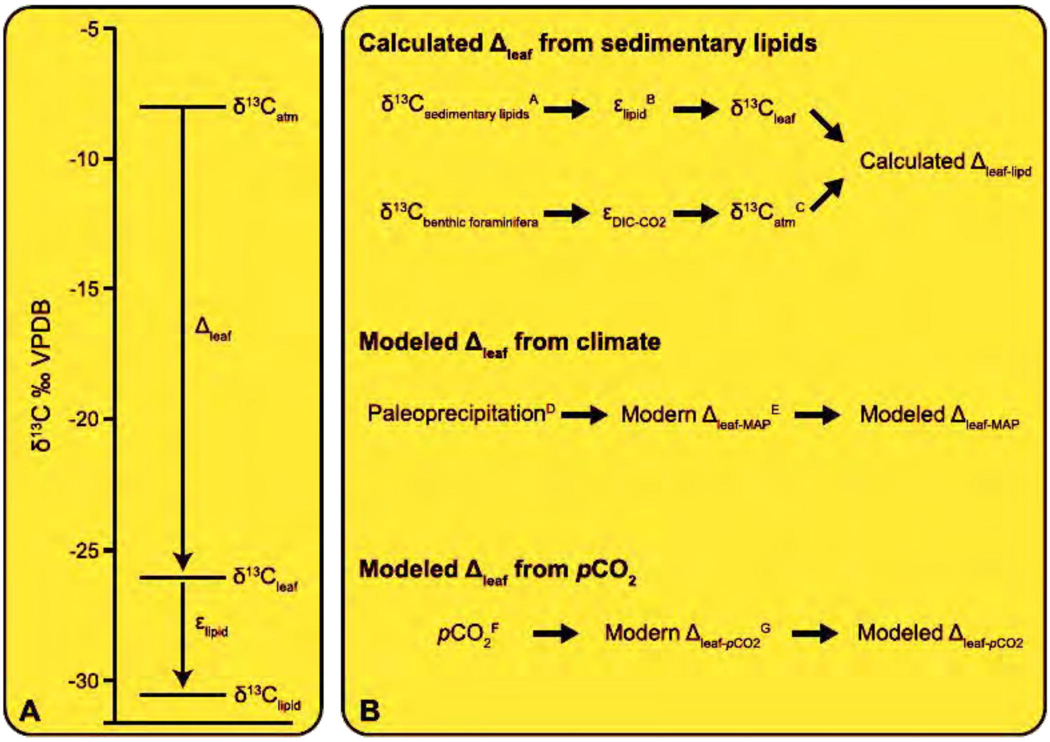


Figure 18: The scheme of calculation Δ_{LEAF} from measured data and the model calculations (B) and of fractionation (A) (Diefendorf et al., 2015).

The palaeoprecipitation used for calculating $\Delta_{LEAF-MAP}$ model were taken from CLAMP analysis by Herman et al. (2002). The recent MAP were taken from Metoffice (2016b) and Metoffice (2016a). The formula used for calculating $\Delta_{LEAF-MAP}$ is taken from Diefendorf et al. (2015) – see formula (3).

$$\Delta_{LEAF-MAP} = 3.14(\pm 0.39) \cdot \log_{10}(MAP) + 11.58(\pm 1.23) \tag{3}$$

This formula can be used for modelling $\Delta_{LEAF-MAP}$ of angiosperms. There is another formula for conifers by Diefendorf et al. (2015) – equation (4).

$$\Delta_{LEAF-MAP} = 5.38(\pm 0.76) \cdot \log_{10}(MAP) + 3.16(\pm 2.18) \tag{4}$$

To calculate Δ_{LEAF} from measured data, formula (5) was used. The atmospheric δ^{13}C was taken from Mackenzie and Lerman (2006).

$$\Delta_{LEAF} = \frac{\delta^{13}\mathrm{C}_{atm} - \delta^{13}\mathrm{C}_{leaf}}{1 + \left(\frac{\delta^{13}\mathrm{C}_{leaf}}{1000}\right)} \tag{5}$$

The atmospheric δ^{13}C was calculated using formula (6) (Arens et al., 2000) for each sample.

$$\delta^{13}\mathrm{C}_{air} = \frac{\delta^{13}\mathrm{C}_{plant} + 18.67}{1.10} \tag{6}$$

The calculated $\Delta_{LEAF-bulk}$ from bulk data were compared with $\Delta_{LEAF-corrected}$ from each n-alkane, corrected from the influence of the fractionation (formula (7)).

$$\epsilon_{lipid} = \left(\frac{\delta^{13}\mathrm{C}_{lipid} + 1000}{\delta^{13}\mathrm{C}_{leaf} + 1000} - 1 \right) \cdot 10^3 \tag{7}$$

5 Results

5.1 Cuticular data

5.1.1 Recent halophytes

The cuticles of recent salt marsh plants and mangroves are presented in Plate 2. Because the fossil samples are mainly shrubs or small trees, there are only a few recent species which can be compared. The salt tolerant or directly halophyte trees are nowadays growing in mangroves.

Therefore Plate 2 a) and b) show the stomata and multicellular gland complexes for salt elimination of *Rhizophora*, which grows in the lowest part of mangroves. The cuticle is massive and thick, the number of stomata is high. Stomata are present only on the abaxial side of the leaf. The other cuticle of a mangal species is shown in Plate 2 c) and it is *Laguncularia*. The cuticle is also very thick, the areas for elimination of salt are not present, but the number of stomata is also very high. Both of these mangal trees are adapted for higher salinity and this adaptation is reflected also in the cuticle thickness and number of stomata.

Plate 2 d) shows a cuticle of *Salicorina europaea*, a plant growing in the low, middle and sometimes also in the high salt marsh zone. This herb has very thin cuticle covered by a thick layer of wax. The wax layer is completely covering and protecting the cuticle which has a high number of stomata. No trichomes were observed. *Salicornia europaea* is classified as a salt tolerant plant and it grows in the soils with high water level and high fluctuations of salinity during the year. It grows in the zone furthest from the creeks where oxygen level is low and evaporation rate high (Long and Mason, 1983).

Plate 2 e) presents the cuticle of the salt marsh plant *Triglochin maritima*, which grows only in the middle zone of the salt marsh. The trichom-like structures are present around the stomata and the stomata are lined up in a regular striped pattern. The cuticle is not very thick and it is covered by a thin layer of wax, unlike that of *Salicornia.*

Lastly, Plate 2 f) shows a cuticle of *Halimione portulacoides*, which grows in low and middle zones of salt marshes. The epidermal cells are very different from all other samples; these cells are probably protecting the leaf and the very small stomata are submerged under this layer of cells. The stomata are 2 or 3 times smaller than the stomata of

Salicornia or *Triglochin.* The leaves of *Halimione* are seemingly very hairy, but under the microscope, it is possible to see that this hairy appearance is caused mainly by the special protective cells, which cover the entire leaf. *Halimione portulacoides* grows in soils with low water level and stable salinity around the creeks (Long and Mason, 1983) and the rounded cell structures on the leaves might be an adaptation for this conditions.

Plate 3 shows *Salicornia europaea* under SEM. One can see the thick waxy layer protecting the whole plant and the surface roughness quite similar with *Pseudoasterophyllites* in Plate 6 c). This might indicate the zone where *Pseudoasterophyllites* may have grown in the palaeomarsh.

Plate 4 presents *Triglochin maritima* under SEM. The trichom-like structures seen in the light microscope are not present under SEM, therefore the trichoms are probably not present. Stomata are massive and ordered in regular stripes in regular pattern.

Plate 5 shows the leaf surface of *Halimione portulacoides* built of by a thick layer of rounded cells which are protecting the submerged stomata. Stomata are hardly seen in between the rounded cells, but in the light microscope they are clearly present (see Plate 2, Fig. f)).

5.1.2 Fossil material

The cuticle structures are often very important for determination of taxa or of the palaeoenvironment. The anatomy of stomata, the thickness of the cuticle and the density of stomata is a very good indicator of the environment. Also some other structures on or in the cuticle, trichoms, trichom's base, hydatods, structures for extraction of crystals of minerals (salt) can occur. The halophytes in mangrove ecosystems can have thick cuticles and extract the salt crystals.

The cuticles of the fossil samples are shown in Plate 6, where the difference in the thickness and massiveness of cuticles of different species is clearly visible. Lauriod angiosperms have a very thin abaxial cuticle and it is not unusual that during the maceration this cuticle is totally dissolved. Therefore it is necessary to be careful in the process of maceration in the case of lauroid angiosperms and to pay attention to this thin cuticle, otherwise the result of the maceration would be just the adaxial cuticle without any stomata.

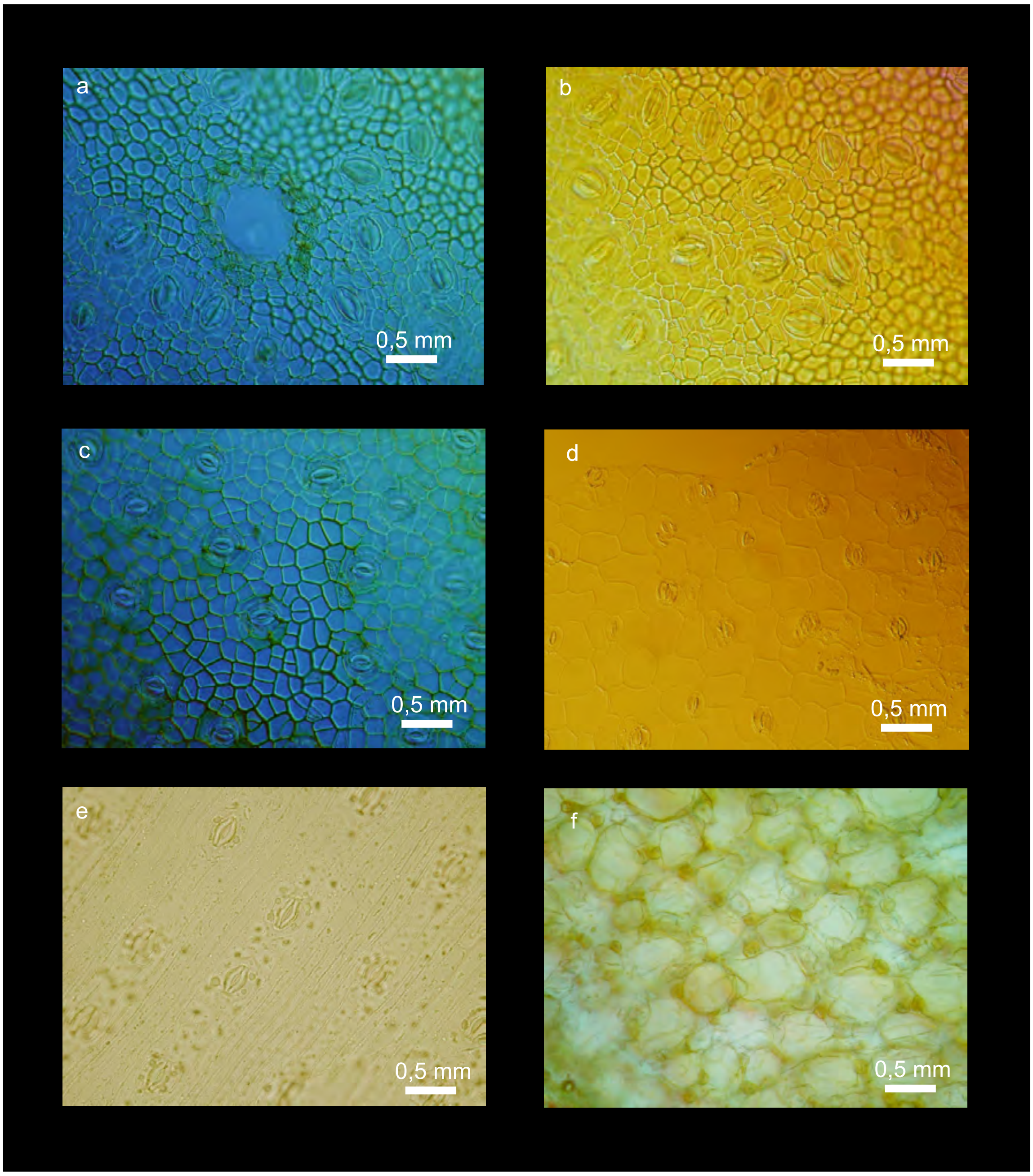


Plate 2: Cuticles of the recent salt marsh and mangrove plants: a) and b) stomata and area of salt excretion of *Rhizophora* from lowest mangrove zones, Everglades, c) Stomata of *Laguncularia* from Everglades, d) Stomata of *Salicornia europaea* from salt marsh, GB, e) Stomata and trichom structures of *Triglochin maritima* from salt marsh, GB, f) Very small stomata of *Halimione portulacoides* from salt marsh, GB.

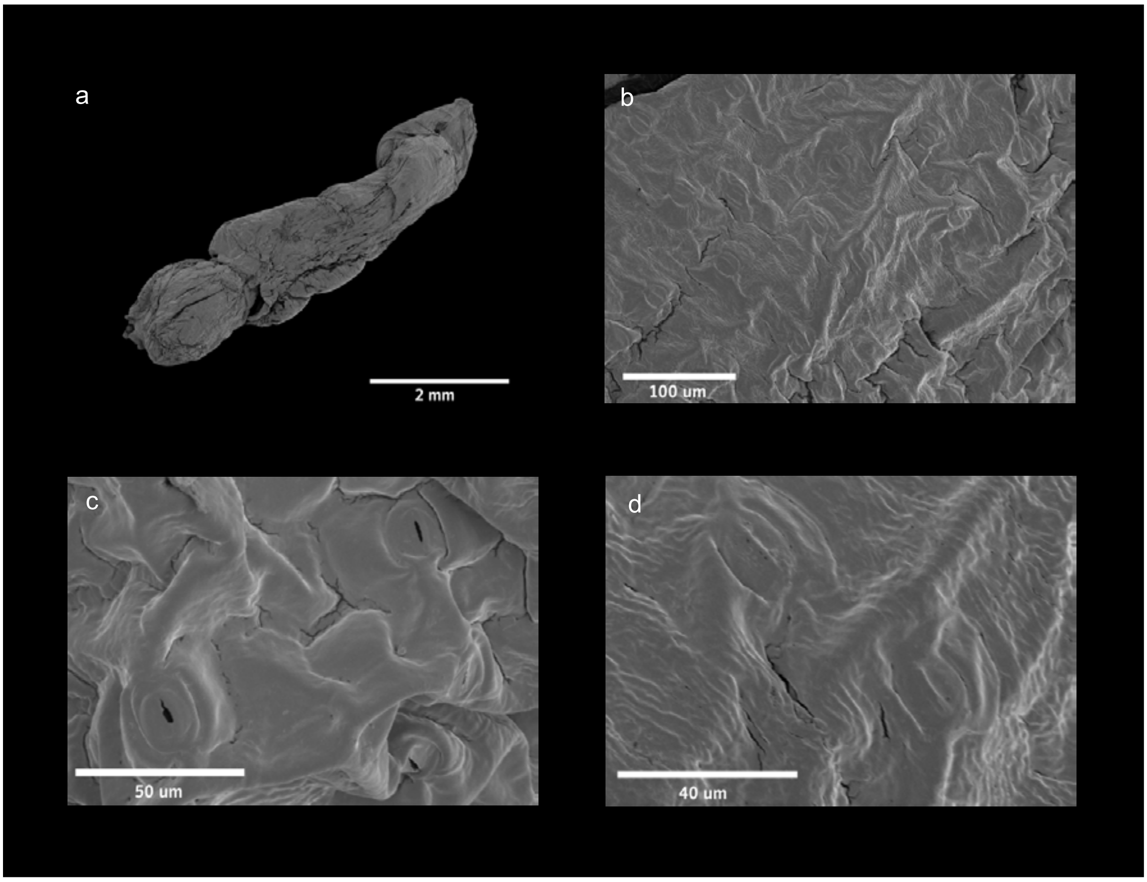


Plate 3: *Salicornia europaea* under SEM, a) whole branch of *Salicornia europaea*, b)–d) external surface of leaf with stomata covered by thick layer of wax.

In Plate 6, Figures a) and b) show the abaxial cuticle with stomata. In the case of a) it is also possible to see trichom's base. The cuticles and the number of stomata in the case of lauroid angiosperms might represent a non-halophytic plant growing in floodplains.

Plate 6 c) shows the cuticle of the angiosperm herb *Pseudoasterophyllites*. Its cuticles are not particularly thick (3–7 µm), the number of stomata is not very high and there is no evidence of trichoms. This herb was probably growing in the salt marsh as a salt tolerant plant. The cuticle outer surface is similar to the cuticles of recent herbs growing in the salt marsh like *Salicornia* (see Plate 2 and Plate 3).

The figure d) in Plate 6 shows a cuticle of *Ginkgo huttonii*, which is classified as a non-halophytic tree growing probably in the floodplain as well as the lauroid angiosperms. Ginkgoales have generally thick cuticle and the maceration takes a long time. Stomata are present only on the abaxial side of the leaf and the number of stomata is comparable with lauroid angiosperms.

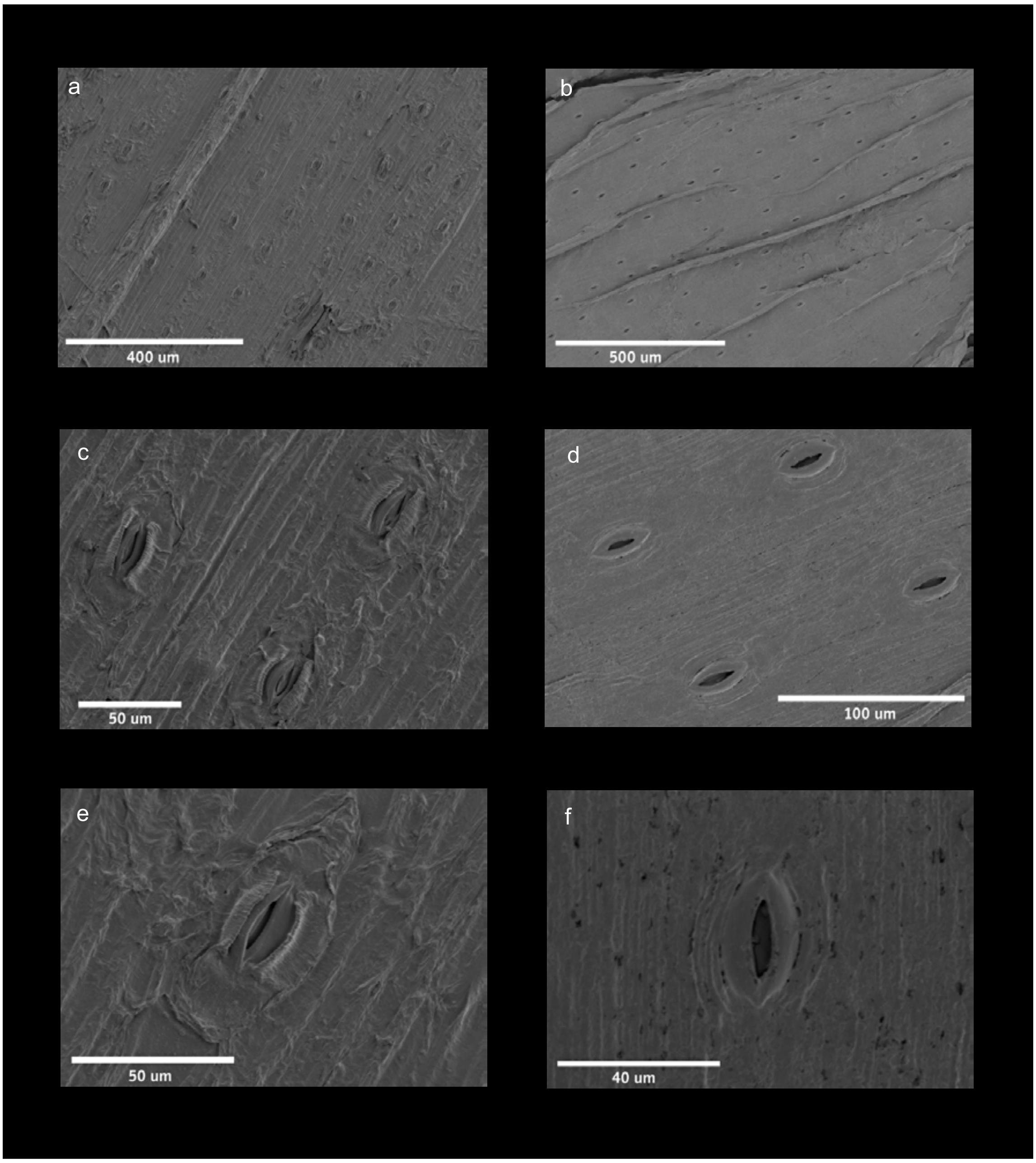


Plate 4: *Triglochin maritima* under SEM, a), b), d) and f) Cuticle with stomata, external surface, no trichoms are present, c) and e) internal surface of leaf with stomata.

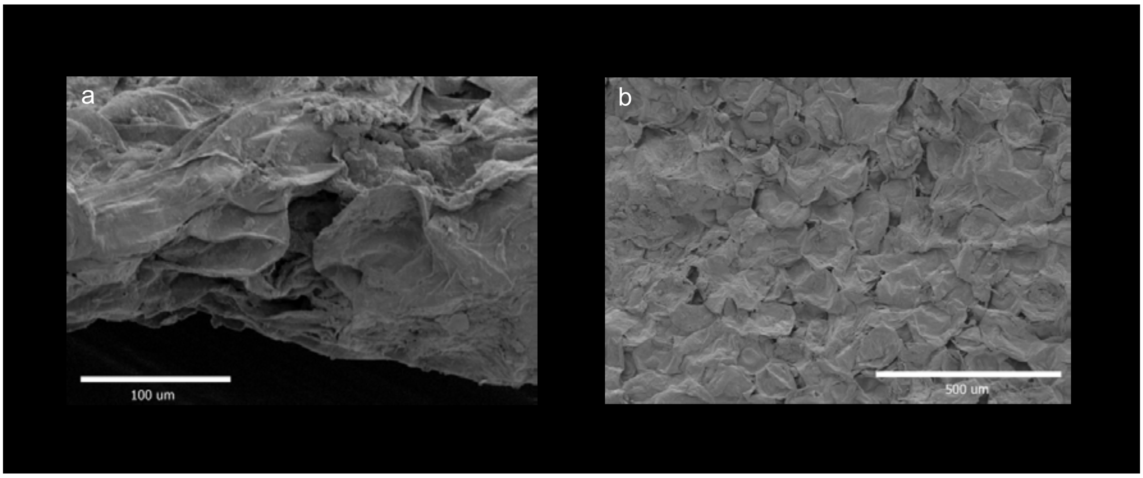


Plate 5: *Halimione portulacoides* under SEM, a) and b) external surface of leaf with submerged stomata; the protective layer of rounded cells is covering the stomata.

The last two figures of Plate 6 are cuticles of *Tritaenia* which is assigned to Ginkgoales by Kvaček (2016) and therefore the cuticle is very thick and massive as well as in the case of *Ginkgo huttonii*. There is no occurrence of trichoms, but *Tritaenia* has very characteristic papillae which are better seen in Plate 7. The number of stomata is higher than in the case of *Ginkgo huttonii* and therefore we can think about its growth in areas with lower water availability.

Plate 7 shows the cuticle of the abaxial side of a *Tritaenia* leaf under SEM. As mentioned above, *Tritaenia* has very characteristic papillae around each stoma. Stomata are arranged in belts running parallel to the leaf margin (see Fig. b) in Plate 7) and the stomata are not organized within the belt. The stomatal pattern is most similar with the pattern of recent plant *Triglochin* (see Plate 2, Fig. e)). Therefore *Tritaenia* might grow in a similar zone of the palaeomarsh as is today's middle marsh zone, based on comparing the stomatal pattern. The ginkgoalean plants usually have a thick cuticle, as well as *Tritaenia*.

Plate 8 shows the abaxial cuticle internal surface of *Eretmophyllum* under SEM. *Eretmophyllum* is assigned to Ginkgoales. The stomata are not ordered in any pattern, they are randomly placed on the leaf. The arrangement of the stomata is not very useful in case of comparing with the recent salt marsh or mangal plants. But in combination with size of the stomata, it might grow in the "salicornia zone", what is the zone the furthest from the creek with high water level and very high fluctuation of salinity during

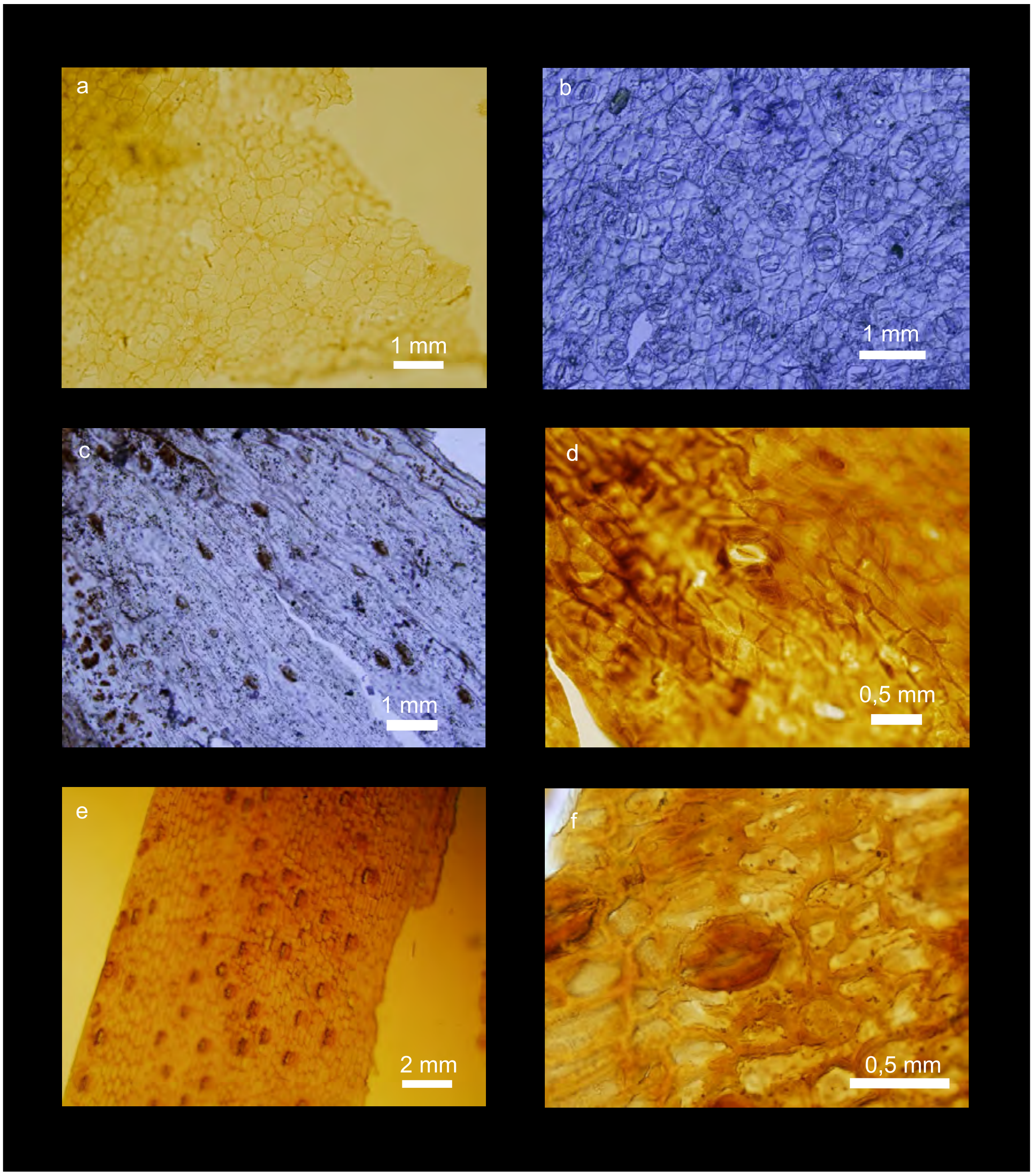


Plate 6: Cuticles of the fossil samples: a) and b) stomata and trichom's base of lauroid angiosperms from Pecínov and Malá Chuchle, respectively, c) Stomata of *Pseudoasterophyllites* from Pecínov, d) Stoma of *Ginkgo huttonii* from Scalby Mills, Yorkshire, GB, e) and f) Stomata of *Tritaenia* from Duingen, Germany.

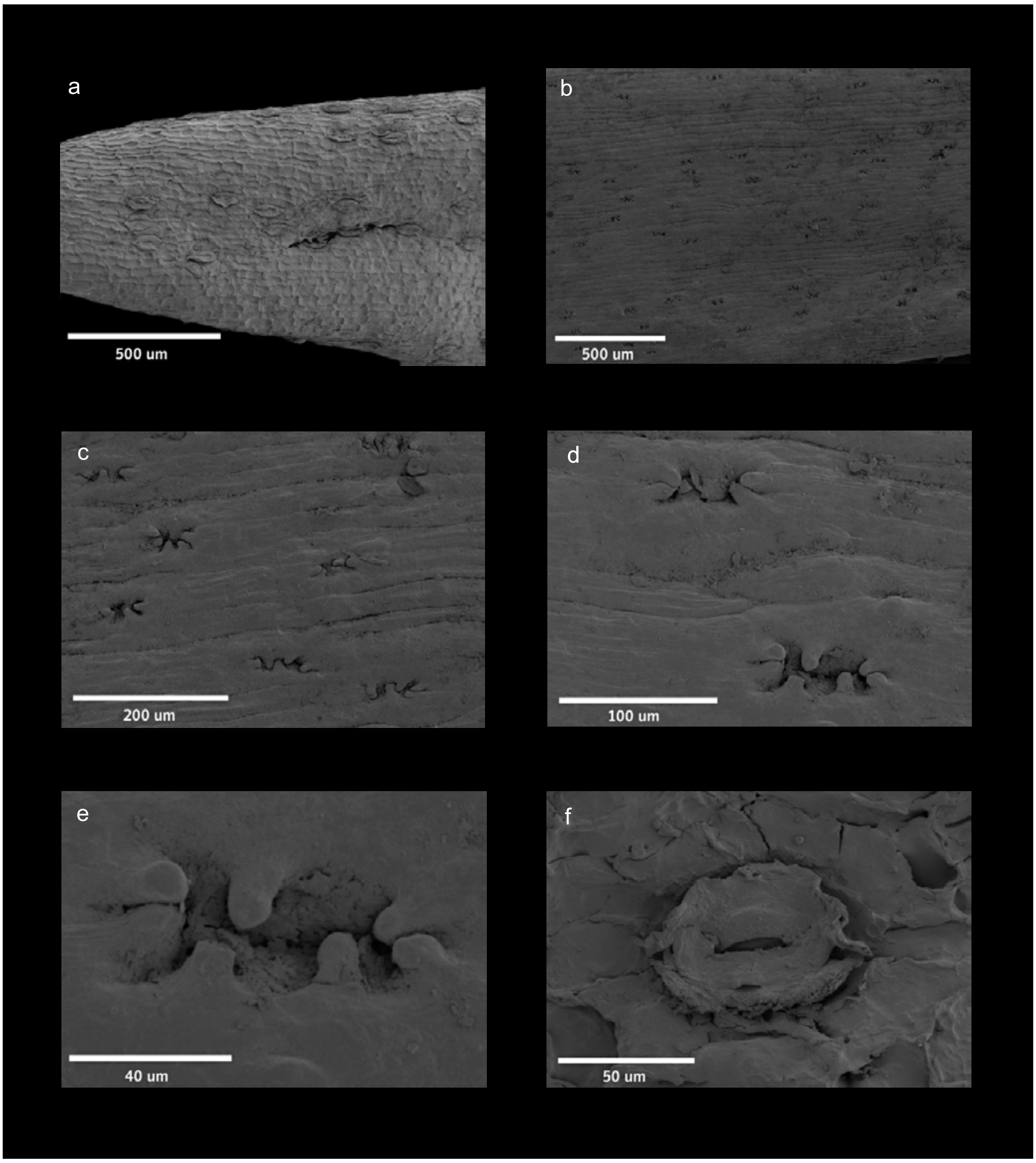


Plate 7: SEM photos of *Tritaenia*, a) and f) abaxial cuticle with stomata, internal surface, b)–e) abaxial cuticle with stomata and characteristic papillae, external surface.

the year.

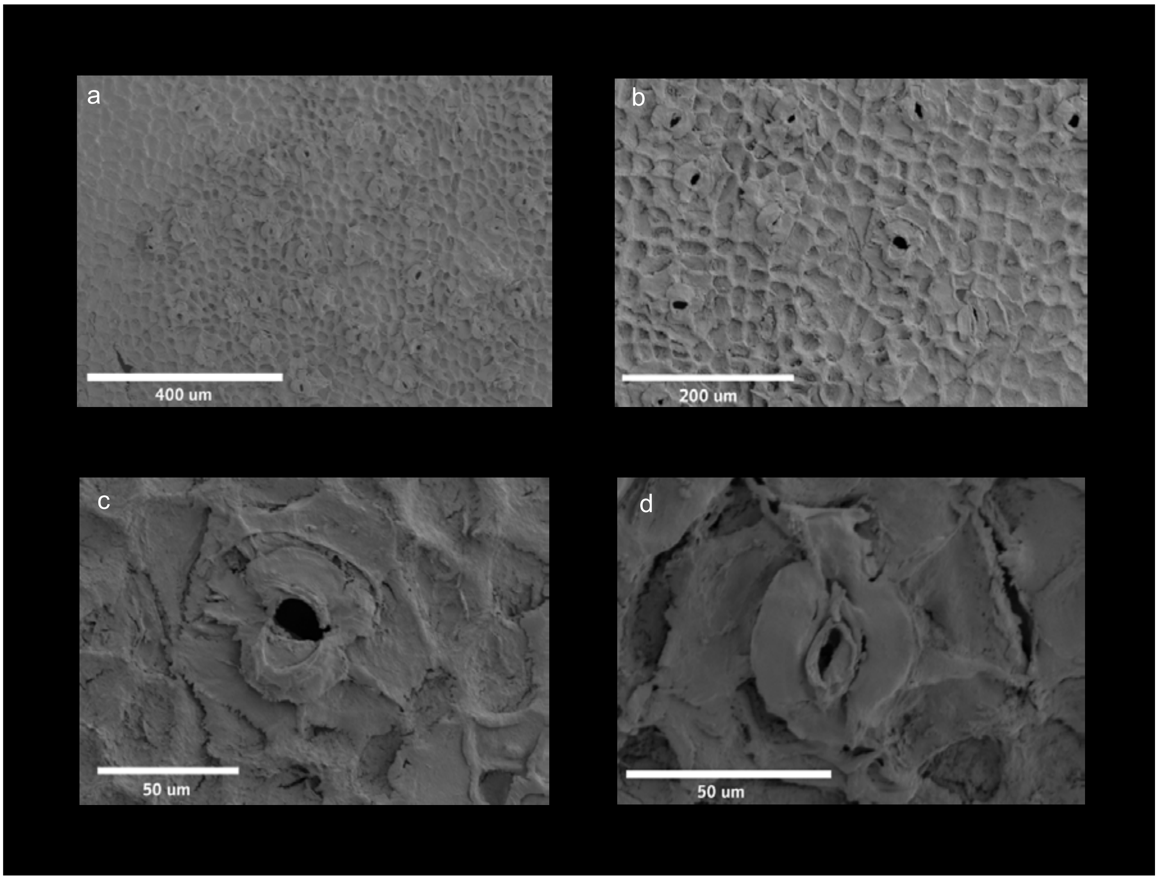


Plate 8: SEM photos of *Eretmophyllum*, a)–d) abaxial cuticle with stomata, internal surface.

5.2 Bulk C results

The measured bulk values are representing the ^{13}C enrichment or depletion in the whole organic matter of the measured samples. Because of the complexity of the plant's body, the values are different in different parts of the plant. But the measured values could represent the average δ^{13}C of the plant. For better correlation of the samples the Compound Specific Isotope Analysis (CSIA) is used, see Section 5.3.

5.2.1 Recent salt marshes

Bulk tissue from the six C3 salt marsh species sampled across the three marshes showed weak gradient connected with distance from the sea. Therefore the hypothesis about the gradient connected with the distance from the shore is not supported and the model is more complicated. Measured data are proving the mosaic model of distribution in the salt marsh and data are following the distribution model with dominant species, which are adapted for salinity variations or stability, content of O_2 in the soil and the water availability (see Section 3.1.2). Plants growing further away from the creek are more adapted for extreme conditions and are better buffering the salinity variation. In general, the measured recent samples are corresponding mainly with water availability as is possible to see on plots 19, 20, 21.

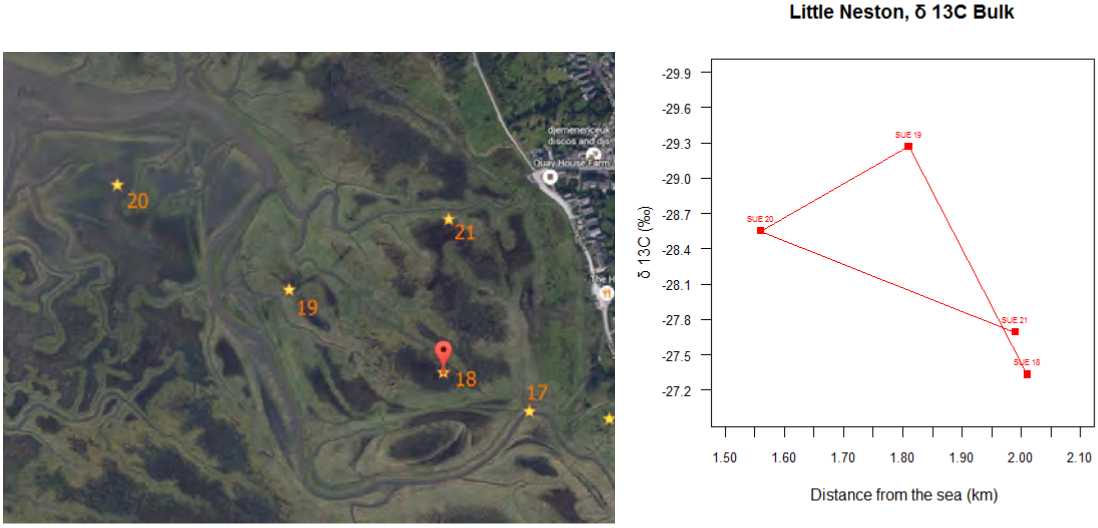


Figure 19: Plotted δ^{13}C bulk of samples collected in the salt marsh Little Neston according to the distance from the sea. SUE – *Suaeda maritima*. Map: (Google, 2016).

From the marsh in Little Neston, only one species was measured and the distances are not well documented. This marsh represents the high marsh where salinity is not very high. Only during the spring tides the marsh is flooded and the only influx of salt water is the creeks. On the other hand, in this zone, water stress can occur more than salinity stress. Therefore the most water stressed samples are in location 18 and 21 which are in drier parts of the marsh (no. 18) or in the salinity stress – near the creek (no. 21). Locations 20 and 19 are in lower water stress, probably in conditions where more water is available in the soil than in locations 18 and 21.

At the marsh in King's Lynn, four species were collected to find some trends. This marsh represents the middle marsh. Fig. 20 shows that the species have different values of δ^{13}C in the same location. This phenomenon is observed also in the next salt marsh in Grange-over-Sands, but it is possible to find a trend-line. A very clear trend is shown

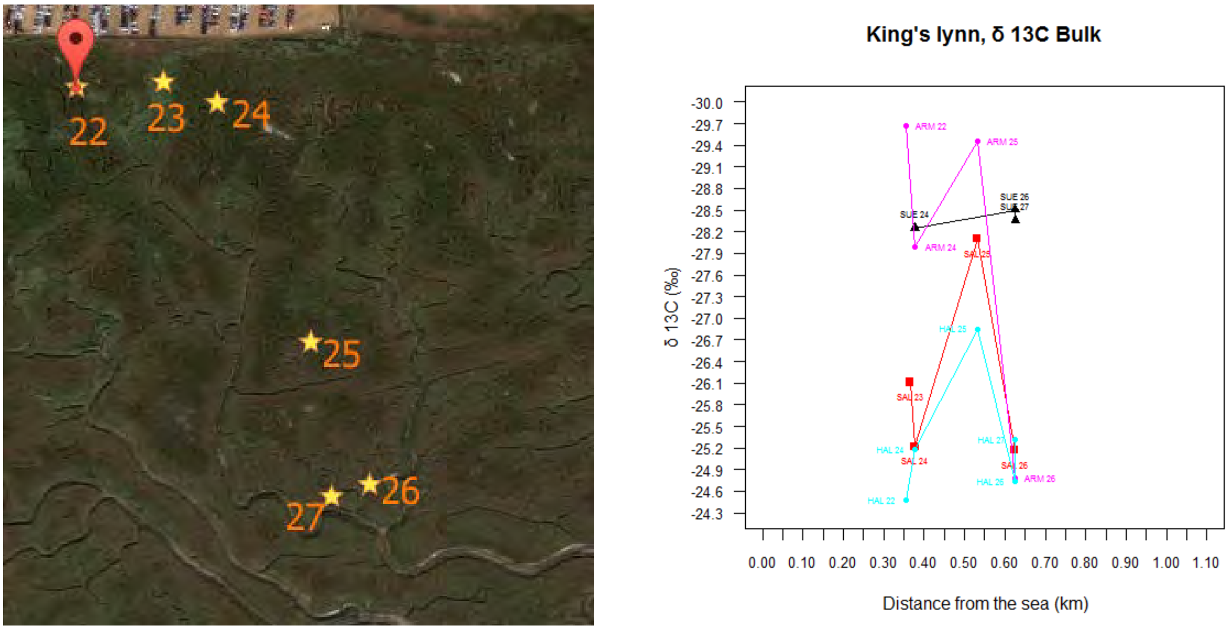


Figure 20: Plotted δ^{13}C bulk of samples collected in the salt marsh King's Lynn according to the distance from the sea. SAL – *Salicornia europaea*, ARM – *Armeria maritima*, SUE – *Suaeda maritima*, HAL – *Halimione portulacoides*. Map: (Google, 2016).

between locations 24, 25 and 26. The samples from location 26 are in low water stress which can be caused by high water availability or more probable is the low salinity. In the case of locations 24 and 26, the plants are growing in higher salinity and therefore they are ^{13}C-enriched compared with the samples from location 25. Locations 26 and 27 have quite similar conditions and therefore the ^{13}C values don't differ very much. From Fig. 20 it is more than clear that the mosaic distribution fits better than the zones. This is shown also in the next salt marsh.

In the salt marsh in Grange-over-Sands, most of the samples were measured and the measured values differ very much between the species, but the values are usually following the same trend. A very interesting thing is shown in Fig. 21, where *Plantago maritima* (blue points and line) has almost inverse trend than *Glaux* and *Salicornia*. These two species have close trend-line, mainly in the lowest part of the marsh. Comparing the values of different species in one location, it is possible to see that conditions in the locations are not similar. In the case of location 36, the values of all measured species are quite close to each other. In contrast, locality 38 has a very wide range of values (from -25.5‰ to 28.1‰). The same phenomenon is observed in locations 34 and 35. Location 33 is

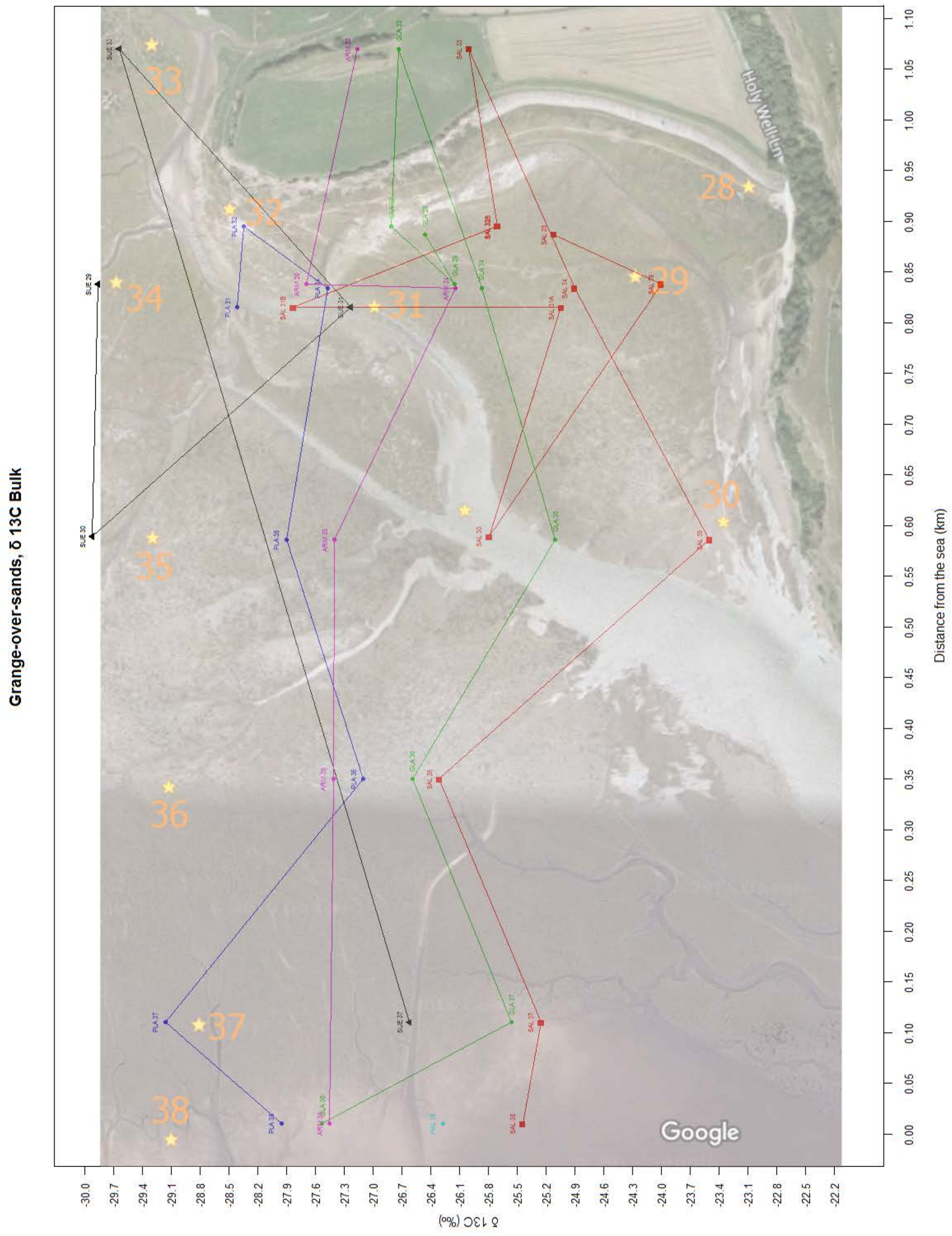


Figure 21: Plotted δ^{13}C bulk of samples collected in the salt marsh Grange-over-Sands according to the distance from the sea. SAL – *Salicornia europaea*, ARM – *Armeria maritima*, GLA – *Glaux maritima*, PLA – *Plantago maritima*, SUE – *Suaeda maritima*, HAL – *Halimione portulacoides*. Map: (Google, 2016).

interesting for the very close values of *Salicornia, Glaux* and *Armeria*, but the value of *Suaeda maritima* is totally different. Therefore, there is clear influence of more than one factor which is driving with the bulk δ^{13}C values. Location 36 is probably a place with very good conditions and therefore none of the species is in stress.

In location 31, two samples of *Salicornia* were collected. One sample was growing on the edge of a creek (SAL31B) and the second was growing a few meters further away from the creek (SAL31A). The isotopic composition showed in this case that the plant growing on the edge of the creek has low water stress and the salinity stress than the other which is growing further away from the water source and therefore the water stress is higher. Also the evaporation causes higher salinity which is directly connected with the water stress. The difference between these two samples is 2.8‰.

Location 37 exhibits a local gradient or succession of the species. *Salicornia* and *Glaux* grow in water stressed soils, because these species are the most tolerant. *Suaeda* grows in more humid soil, there is lower water stress and probably also the salinity is lower. The less salt tolerant species is *Plantago* which probably grows in the most humid soil where there is very low water stress and low salinity. Then in this locality *Plantago* occupies the "best" conditions and the rest of species are growing in "worse" conditions.

5.2.2 Fossil samples

The method of bulk δ^{13}C is usually used in the fossil record. The method is more often used mainly because of the input amount of sample. For Bulk analysis it suffices to have a very small amount of material and this is a big advantage in the fossil record, because the method is destructive and the fossil material is limited. The results are used for interpretation of water availability and for supporting the palaeoenvironmental reconstruction created from more than one proxy.

The problem with this method is about the wide range of different δ^{13}C composition in every part of the plant. The measured data represent the δ^{13}C of the whole organic matter. One of the goals of this thesis is to find how different these Bulk data are from the compound specific data.

The measured data are presented in Fig. 22, where all samples are shown in one plot and then each locality in a separate plot. The plot with all fossil samples is important

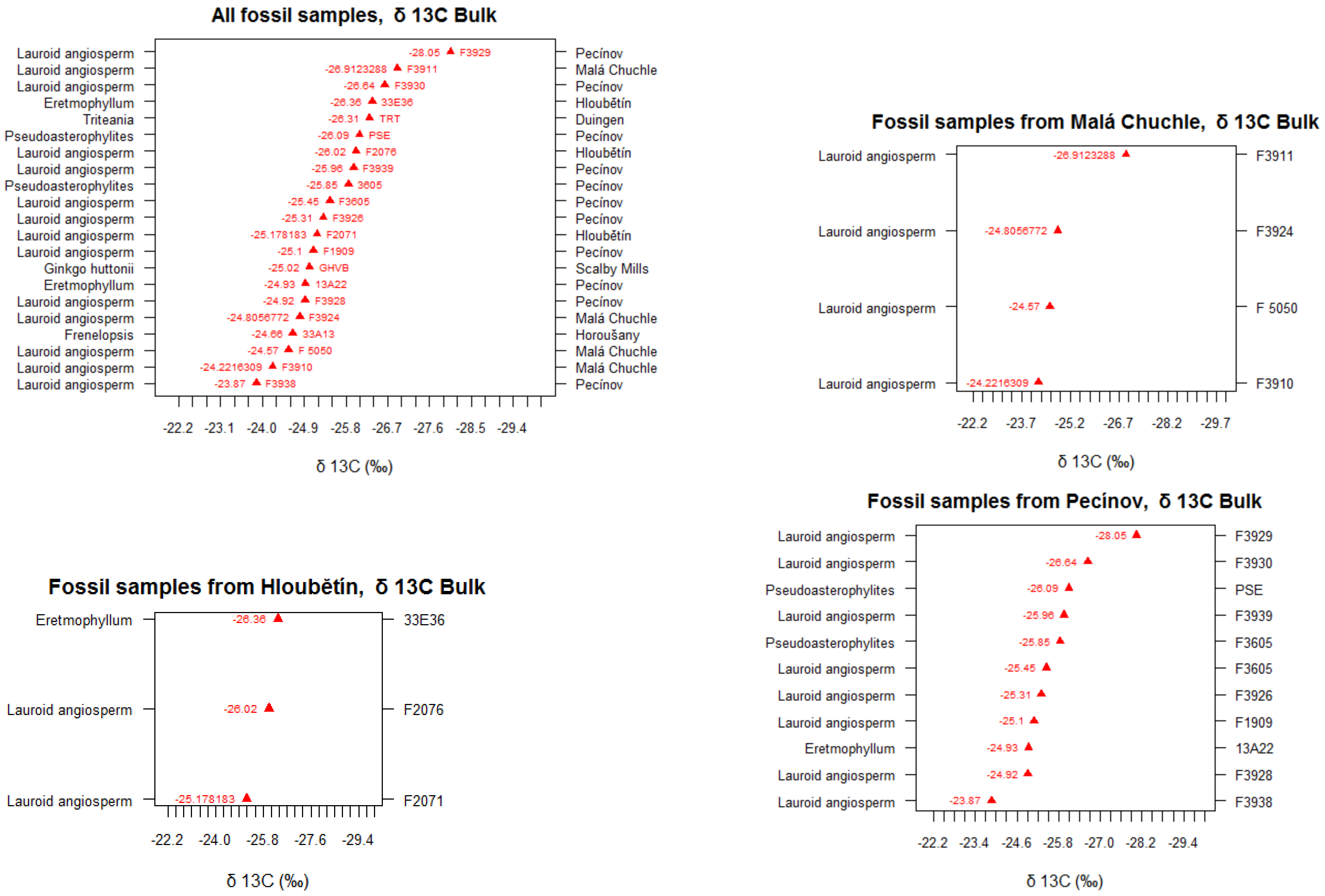


Figure 22: Plotted δ^{13}C bulk of fossil samples from the Cretaceous localities Pecínov, Malá Chuchle and Hloubětín.

for interpreting the species *Tritaenia* from locality Duingen. *Tritaenia* was presented as a non-halophytic plant growing in marginal marine environment (Manum et al., 1999). The assumed environment was a high marsh zone with good water availability (Kvaček, 2016). The isotopic analysis supports this assumption as it is seen in the summarizing plot in Fig. 22. Values of δ^{13}C bulk of *Tritaenia* are lower than values of assumed plants of salt marsh as is *Frenelopsis* or *Eretmophyllum.* These two plants were proven as halophytic plants or salt tolerant plants growing in middle or low marsh zones. In the case of the mosaic model, we can find some similarities with recent *Salicornia* or *Glaux*, the salt tolerant species.

Surprisingly *Ginkgo huttonii* has very high isotopic values which were not assumed, because *Ginkgo huttonii* is presented as a non-halophytic plant growing on flooding planes. Other results which were not supposed are the isotopic values of *Pseudoasterophyllites*, the herb which was compared with *Salicornia* in the cuticular analysis. The δ^{13}C bulk data are lower than was supposed.

Also an interesting result is the value of *Eretmophyllum* from locality Hloubětín, because *Eretmophyllum* is presented as a salt marsh plant, even it was probably growing in higher marsh with lower salinity (Kvaček et al., 2005), but the measured values are equivalent to non-halophytic Lauroid angiosperms growing on the edge of river systems. Both samples of Lauroid angiosperm from locality Hloubětín have more saline character than the *Eretmophyllum.* There is an alternative interpretation that the Lauroid angiosperms were suffering by drought in comparison with the saltmarsh *Eretmophyllum.*

The plots for each locality show the relative relationship between the measured samples and also the possible zonation. In the case of Malá Chuchle only the Lauroid angiosperms were measured and the values have a more halophytic character than was supposed. It can also be the effect of low water availability rather than just salinity that is causing these high values of δ^{13}C bulk. The very same phenomenon is shown in the plot for locality Hloubětín, where the salt tolerant *Eretmophyllum* has lower values than the Lauroid angiosperms which are supposed as non-halophytic plants. In the case of locality Pecínov, there are better results of *Eretmophyllum*, *Pseudoasterophyllites* has lower values than is supposed for a salt tolerant plant and the Lauroid angiosperms are creating a very interesting phenomenon – the values tell us that if this environment was a salt marsh (based on sedimentary records), the Lauroid angiosperms were growing in all of the salt marsh zones. To test this hypothesis it is recommended to see the results of the Compound Specific Isotope Analysis, where we have certainty that the δ^{13}C is really from the fossil plant matter, because as is mentioned above, the bulk analysis is working with all of the organic matter in the sample.

5.3 Compound Specific Isotope Analysis

The Compound Specific Isotope Analysis (CSIA) has several advantages over bulk. As it was mentioned above, this method is measuring δ^{13}C specific compounds which are produced by plant metabolism during its life. The most stable and resistant compounds are cuticular waxes which are compound from *n*-alkanes. Because of the plant fractionation, the isotopic composition of these compounds is reflecting some environmental conditions limiting the plant. The *n*-alkanes have one more advantage and it is the resistance against diagenesis (Diefendorf et al., 2011). Therefore the measured data by GC-IRMS gave us the values of isotopic composition of *n*-alkanes from the cuticular waxes and we can interpret the relative water availability of each sample – even the fossil samples.

5.3.1 Recent salt marshes

In the three salt marshes in GB six plant species were collected in almost all localities (see Fig. 16). This fact is quite interesting because of the salt marsh zonation. As one can observe also in bulk measurements (Fig. 19–21), the species which are indicative of different zones have been present also in the zones where they are not expected to grow. Therefore the mosaic distribution is more supported by these measurements as can be seen in Fig. 23, 24, 26, 27 and 25.

The first figure (Fig. 23) shows the plotted data measured by GC-IRMS. The results are presented as *n*-alkanes, the most common and the most used are C_{23}, C_{25}, C_{27} and C_{29} because leaf waxes have predominantly an even number of carbon atoms (Killops et al., 2005b). The salt marsh in Little Neston is the high marsh zone, but we can see the mosaic distribution of environmental conditions on *Suaeda maritima.* No gradient towards the sea shore was found and the trend-line of *Suaeda maritima* is in the case of all four alkanes almost the same. It is possible to see a shift of measured δ^{13}C values for each alkane.

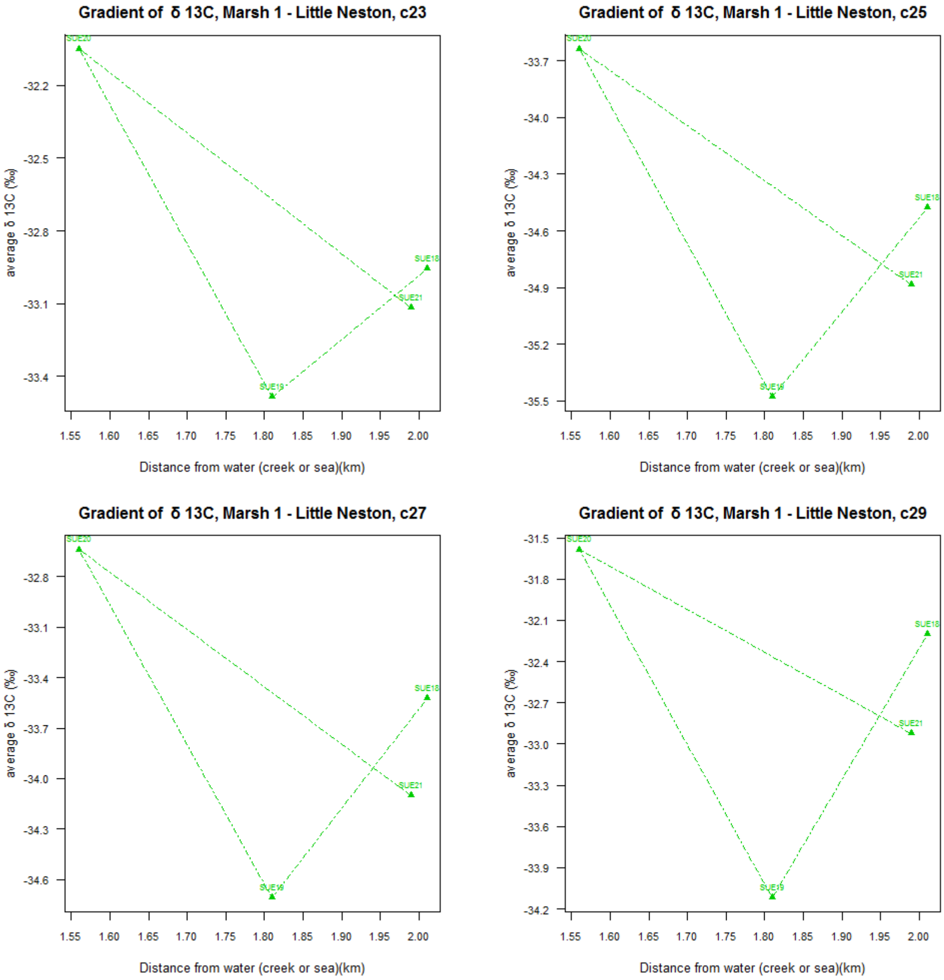


Figure 23: Plotted $\delta^{13}C$ n-alkanes of recent samples from the salt marsh Little Neston. SUE – *Suaeda maritima*

Figure 24 shows the plotted data from the salt marsh in King's Lynn where four species were collected and measured. In the first two plots (C_{23} and C_{25}) *Armeria maritima* is missing. The signal for these two alkanes was not present in the result spectrum. In the case of *Salicornia europaea* it is possible to see a decreasing trend-line which can be presented as the rising distance from the sea, the water availability is lower and the salinity could be higher. The measured values are following this trend in all tree plots. Three other species have more complicated trend-lines and this could be caused by connection of the species on the creeks. *Halimione portulacoides* is growing in areas around the creeks, therefore the values are very oscillating due to the different distance from the nearest creek. The plots of C_{27} and C_{29} are supporting the mosaic distribution of plants (environments) in the salt marsh.

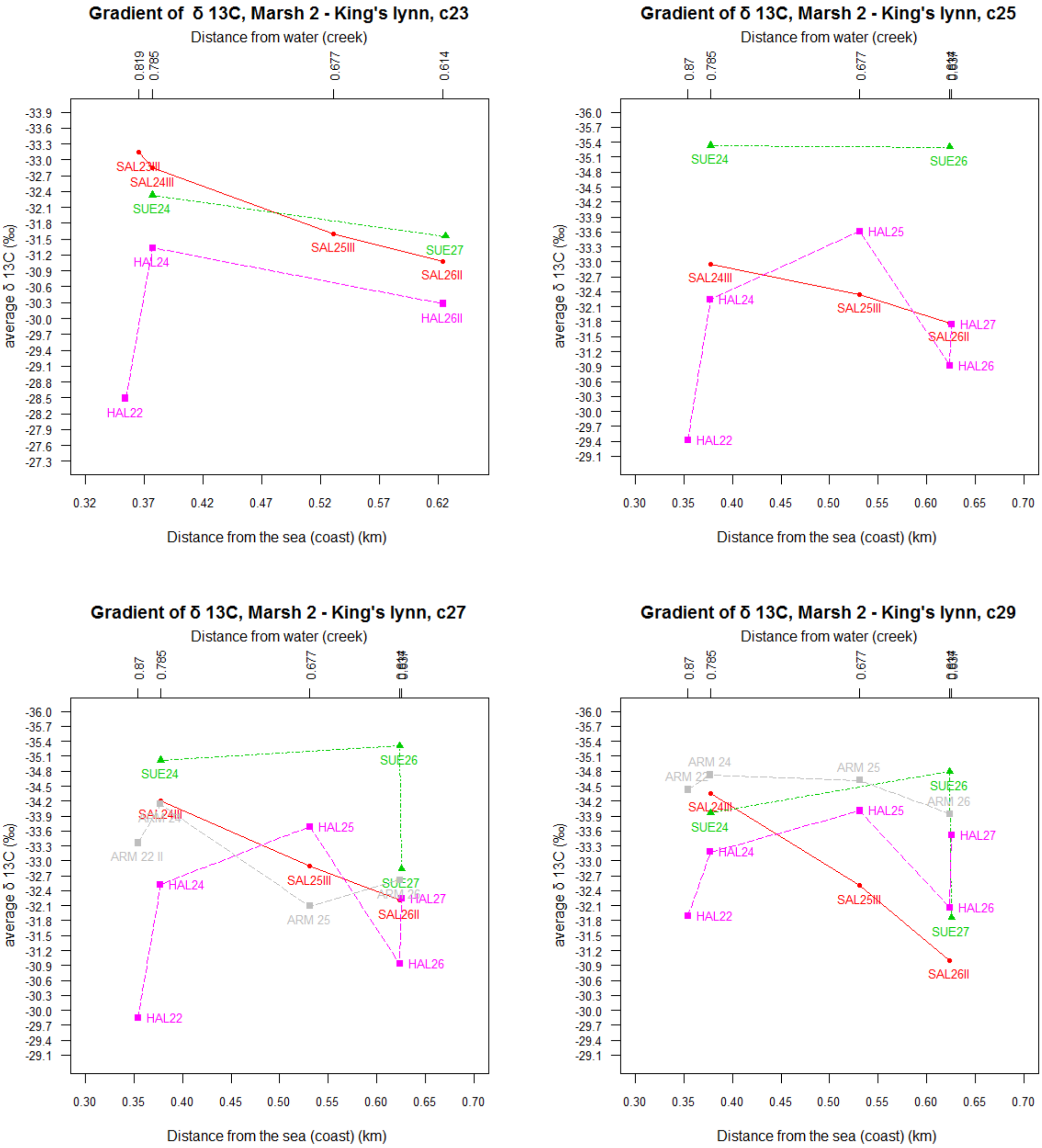


Figure 24: Plotted δ^{13}C *n*-alkanes of recent samples from the salt marsh King's Lynn. SAL – *Salicornia europaea*, ARM – *Armeria maritima*, SUE – *Suaeda maritima*, HAL – *Halimione portulacoides*

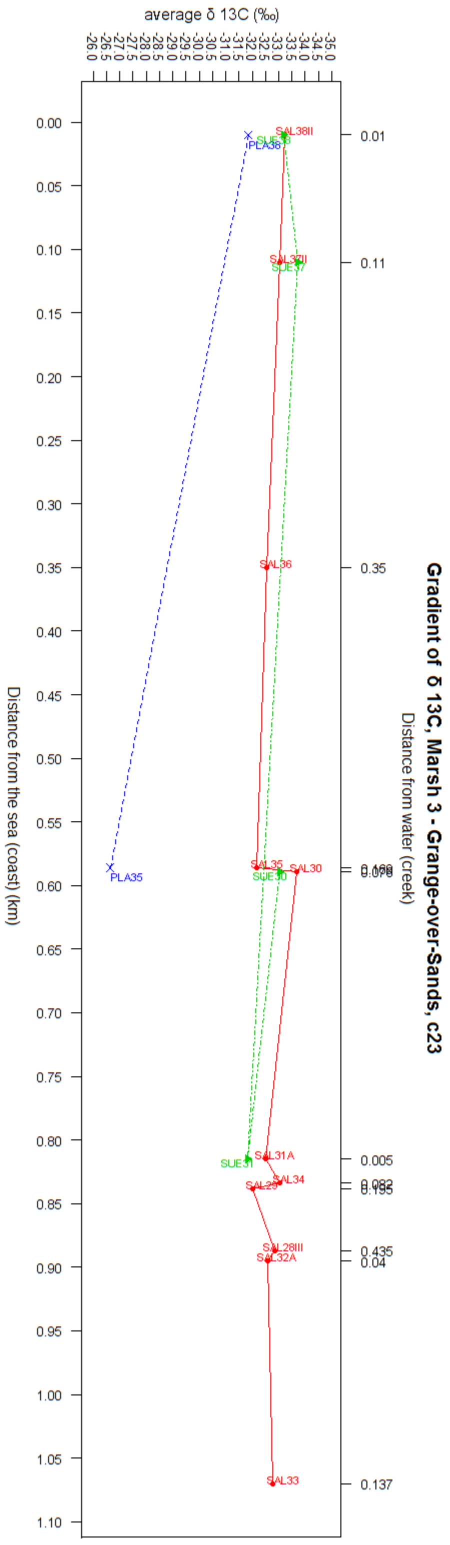


Figure 25: Plotted δ^{13}C *n*-alkanes (C$_{23}$) of recent samples from the salt marsh Grange-over-Sands. SAL – *Salicornia europaea*, ARM – *Armeria maritima*, GLA – *Glaux maritima*, PLA – *Plantago maritima*, SUE – *Suaeda maritima*, HAL – *Halimione portulacoides*

The most complicated situation is shown in the plots from the salt marsh in Grange-over-Sands. Here, the sampling was the most detailed and there were suggested gradients from the nearest creek (tidal channel) or from the sea shore. As the plots show, no clear gradient is possible to follow. There is a slight trend of increase of δ^{13}C values in the plot of C_{23} (Fig. 25) in between the sample 38 and 31 connected with the rising distance from the sea. This trend cannot be distinguished in the next *n*-alkanes plots, but locally it is possible to see trend of abrupt change of δ^{13}C caused by the different distance from the creek. This phenomenon was tested on *Salicornia europaea*, when there is sample directly from the edge of the creek and another sample which is from around 5 meters distant location from the creek. The values of the sample from the creek are by 2‰ lower than the values of the sample growing further away from the creek (see Table 1). This shows that the plants growing around the creek are suffering less from low water availability than the distant ones.

Sample	δ^{13}C	C	average δ^{13}C	DFW	DFS
SAL31A	−32.658	23	−32.495	0.005	0.815
SAL31B	−35.347	23	−34.756	0.001	0.815
SAL31A	−32.947	25	−32.488	0.005	0.815
SAL31B	−35.772	25	−33.842	0.001	0.815
SAL31A	−32.735	27	−32.185	0.005	0.815
SAL31B	−36.674	27	−34.365	0.001	0.815
SAL31A	−31.300	29	−31.337	0.005	0.815
SAL31B	−35.693	29	−33.738	0.001	0.815

Table 1: Table with δ^{13}C values of two tested samples of *Salicornia europaea* in different distances from the water creek. DFW – distance from water, DFS – distance from sea.

Figure 26 shows the plotted values of δ^{13}C C_{25}, C_{27} alkanes. These two *n*-alkanes, especially C_{27}, are most abundant in the leaf waxes. Almost no gradient can be seen in these cases, but it is very clearly possible to distinguish the higher part of the low marsh. This area has a very mosaic distribution of the vegetation which is driven by the mosaic conditions of the environment.

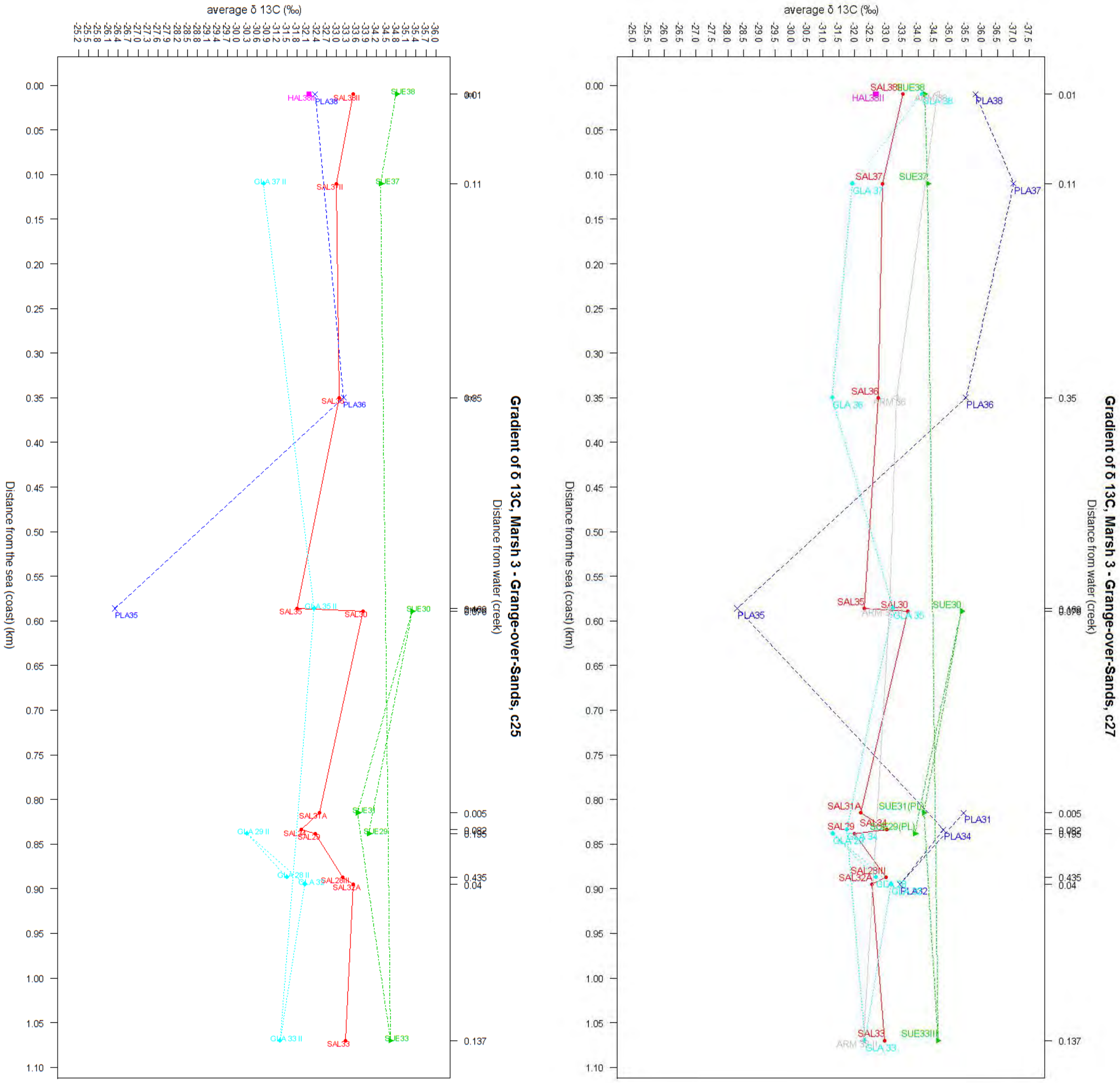


Figure 26: Plotted δ^{13}C *n*-alkanes (C_{25}, C_{27}) of recent samples from the salt marsh Grange-over-Sands. SAL – *Salicornia europaea*, ARM – *Armeria maritima*, GLA – *Glaux maritima*, PLA – *Plantago maritima*, SUE – *Suaeda maritima*, HAL – *Halimione portulacoides*

One interesting thing in these plots is the values of *Plantago maritima.* As is seen, *Plantago* has totally different values than the rest of the species. This can indicate two possibilities: 1) this can be driven by the mosaic environments, that means really different conditions in a very small area, then *Plantago* is choosing the best conditions with high water availability and therefore other species have to grow in the worse conditions with lower water availability or higher salinity or 2) *Plantago* is very well adapted for these conditions, better than other species and therefore is fractionating much more effectively than for example *Salicornia*.

The more probable interpretation is the first one, because as is seen in the plot of δ^{13}C C_{27}, the sample PLA35 has very high values in contrast with SAL35 and GLA35. Then if *Plantago* were adapted for higher salinity than *Salicornia* as it could appear in other localities, then in this very dry or saline conditions of locality 35, it should have lower values than *Salicornia* as it is in all other localities. But because of this really high δ^{13}C in the locality 35, it seems that the first interpretation is correct – *Plantago* is occupying the best conditions (high water availability) and the rest of the species have to grow in worse ones. In the locality 35 it probably happened some abrupt environmental change in the conditions where this *Plantago* grew and the other species are growing in better water conditions or they can better buffer extreme conditions – which is more probable than really different environmental conditions in one place. Therefore, we can use *Plantago* as a species which is sensitive for water availability and salinity changes more than the others species growing in the salt marsh.

The values of measured δ^{13}C C_{29}, C_{31} alkanes are shown in the Fig. 27. In the case of C_{29} alkane, it is seen that this alkane is as important as the previous ones (C_{25}, C_{27}) for the CSIA. These three alkanes are the most used, because they are in high concentration in the leaf waxes. Some other environmental changes are seen in the line of *Plantago* not only the locality 35, which is not as extreme here as it was in the case of C_{25} and C_{27}. The locality 31 is a very perspective locality for *Plantago* – there is low water stress or low salinity and it occupies the best conditions, because comparing with the values of the other species, they are in slight water stress situation.

The highest water stress conditions are observed in the case of *Salicornia* (SAL31A), as can be seen also in Table 1. This is a sample growing around 5 m from the creek and the conditions are really dry and haline. Almost the same phenomenon in the case of *Plantago* is observed in locality 37, where all others species are in water stress conditions in contrast with the *Plantago*. Locality 36 is very stressful for *Glaux maritima* where the values of δ^{13}C are rising up to $-30.5‰$. Therefore it is clear that in this locality *Glaux* is growing in the most saline or dry conditions and all other species are able to buffer these conditions better then *Glaux* (e.g., *Salicornia*) or they are growing a few centimetres away from these conditions, where the water availability is higher (e.g., *Plantago*).

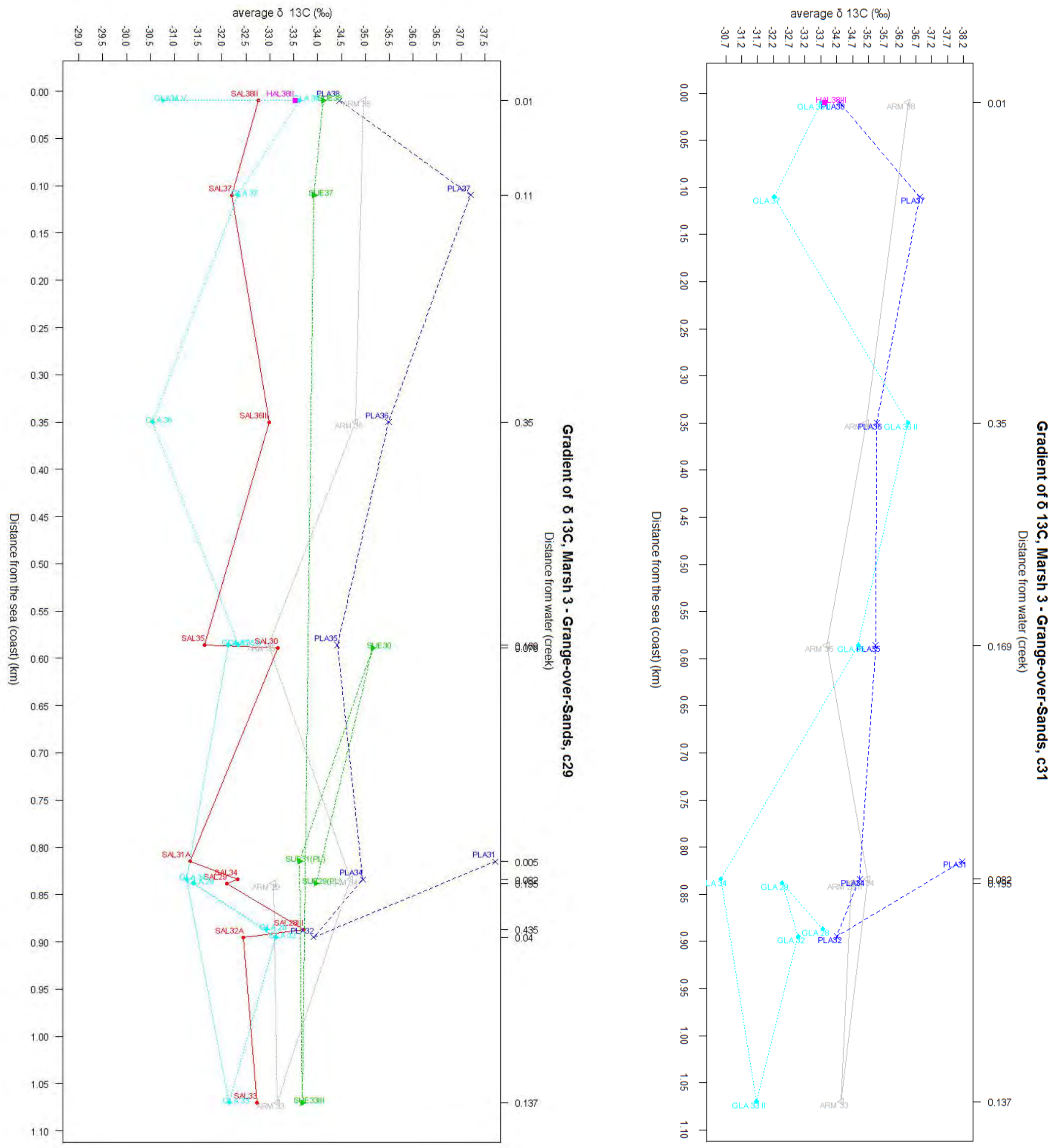


Figure 27: Plotted δ^{13}C *n*-alkanes (C_{29}, C_{31}) of recent samples from the salt marsh Grange-over-Sands. SAL – *Salicornia europaea*, ARM – *Armeria maritima*, GLA – *Glaux maritima*, PLA – *Plantago maritima*, SUE – *Suaeda maritima*, HAL – *Halimione portulacoides*

The values of δ^{13}C C_{31} alkane were present just for four plants. Here one can see a great difference of habitats of *Glaux* and *Plantago*. These two species have almost inverse values except the localities like 38, where their values are quite the same. This locality is very near the sea and the variability of the water availability is probably not high. As it can be seen in all others plots above in this locality the values of all species are very close. They are creating an interval, where the extremes are values of *Salicornia* – the highest water stress, and *Armeria maritima* or *Plantago* – the lowest water stress. Almost the same interval is created in the locality 33, where the highest water stress is in the case of *Glaux* and on the other hand the lowest water stress is in the case of *Suaeda* (*Plantago* is totally missing).

To summarize the results, there is no clear gradient connected with the distance from the sea. The gradient based on the distance from the tidal creek can be observed in small scale as it was done with *Salicornia*. In larger scale, there is just the mosaic distribution of vegetation in the salt marsh and the rate of fractionation is also very important effect which can influence δ^{13}C values. For the plants which are buffering the environmental changes as is *Salicornia*, it is very hard to find some greater trend or gradient. The species which are less salt tolerant or unable to buffer the environmental changes as is *Plantago* or *Halimione*, it is possible to observe the very mosaic distribution where these species occupy the best conditions (high water availability) and the other species cannot compete with them: they grow in the remaining area.

5.3.2 Fossil samples

The main reason for analysing *n*-alkanes is higher information values of the δ^{13}C values measured by Compound Specific Isotope Analysis than the Bulk analysis. As it was mentioned above, in the bulk analysis, all carbon in the sample is measured. In the case of Compound Specific Isotope Analysis (CSIA) only the leaf waxes are measured which are separated before the GC-IRMS processing. The most abundant *n*-alkanes in the fossil samples were C_{27}, C_{25}, C_{29} and also the C_{23}. The results of CSIA are shown in Fig. 28 and 29.

The *n*-alkane C_{23} was not as abundant as the others, but in the samples which have been separated from high amount of material, there was also signal of this alkane.

If we compare the fossil samples relatively in between each other, the water stress can be interpreted. It is necessary to keep in mind that the samples are from different localities. Then *Frenelopsis* is the most water stressed plant. Unfortunately, there is

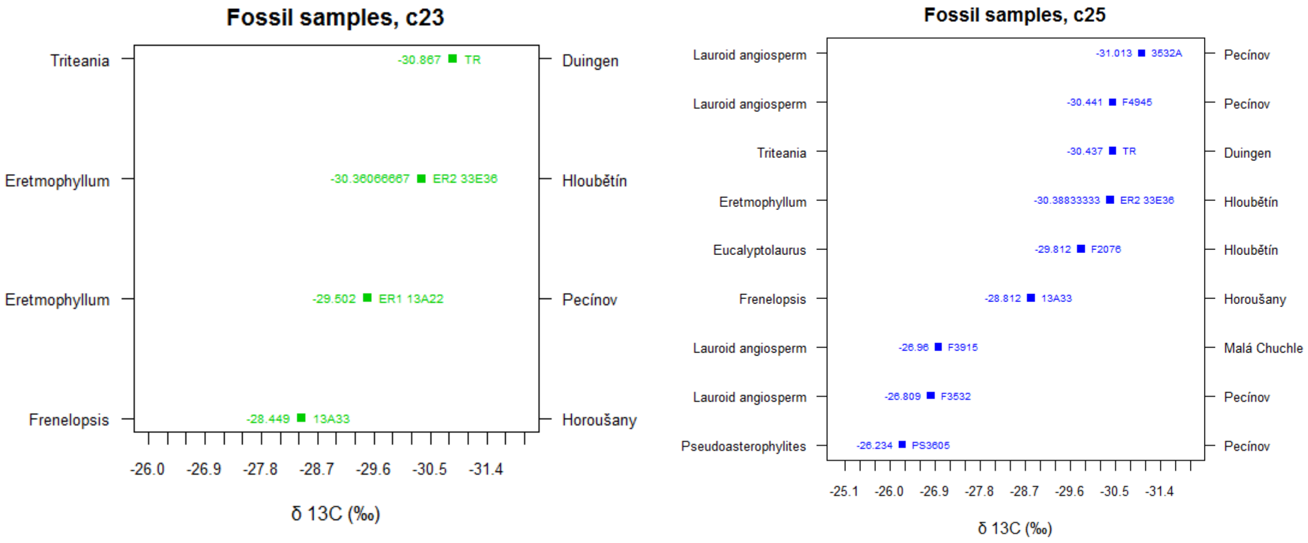


Figure 28: Plotted δ^{13}C n-alkanes (C_{23}, C_{25}) of fossil samples from all the localities – Pecínov, Hloubětín, Malá Chuchle, Horoušany and Duingen.

no other sample measured by CSIA from the locality Horoušany, but as we know from the sedimentary record, *Frenelopsis* is found in layers which are consisted often only of *Frenelopsis*. This indicate monodominant plant association, which could be interpreted as caused by extreme conditions. *Frenelopsis* is reconstructed as a shrub and it is interpreted as a salt marsh plant (Fatka and Kvaček, 2006).

In the case that in these "*Frenelopsis*-rich" layers is found something else than *Frenelopsis*, it is usually *Eretmophyllum*. This ginkgoalean tree is shown in the plot as well and the δ^{13}C values are rather lower than the values of *Frenelopsis*, but not in all cases. In the plot of C_{27} alkane, there are the values of *Eretmophyllum* higher than the values of *Frenelopsis*. Although these two measured species were growing in different localities, *Frenelopsis* is found also in locality Pecínov, but it was not measured in this thesis. Based on these data, it is supposed that these two species are the salt marsh species and they were growing in the conditions where recent *Salicornia* or *Suaeda maritima* grow. *Eretmophyllum* is probably less salt tolerant than *Frenelopsis*, but both plants grow in the low and middle marsh zone with high salinity and low water availability.

Tritaenia from the Lower Cretaceous of Duingen can be assessed as a less salt tolerant plant growing in the middle or high marsh zone with higher fresh water availability. There is another interpretation of the values of *Tritaenia* based on the recent salt marsh plants. *Tritaenia* could grow on the edge of the creeks where water availability is high and salinity low. Generally, *Tritaenia* can be classified as a plant which is not able to buffer the oscillating conditions and therefore the area where it is expected to grow was an environment with stable fresh water conditions (edge of the river, edge of the creeks).

Pseudoasterophyllites is the only herb which has been found and measured in these localities. It was supposed that this plant could have the same habitat as recent *Salicornia*, but as the measured values show in the case of C_{25} , *Pseudoasterophyllites* is a plant growing in less saline environments with good fresh water availability or in the haline environment with very good buffering ability. When interpreting *Pseudoaterophyllites* it is good to keep in mind the recent mosaic distribution of the salt marsh vegetation. The reason why it is found in the sediments of low salt marsh zone is because it probably grew in the same environment as today's *Salicornia.* It occupied almost all microhabitats in the salt marsh, also where the rest of the plants could not grow. Also because it is a herb, the generation cycle is faster than in the case of trees (Ginkgoales) or shrubs (*Frenelopsis*). Therefore *Pseudoaterophyllites* grew in very variable areas of the marsh, haline environment and also very wet conditions. If the conditions were worse, *Pseudoasterophyllites* has very high values, in contrast to the shrubs and trees, they had to buffer the haline conditions, they had survived also because of their longer generation cycle. Then when the conditions became better, *Pseudoasterophyllites* values are lower and the trees and shrubs would have small changes of the values in comparison with *Pseudoasterophyllites.* As it is seen in plot 29, *Pseudoasterophyllites* has three different values, therefore it is supposed that it grew in all microhabitats of the salt marsh as today's *Salicornia.*

The Lauroid angiosperms (including *Ecalyptolaurus*) are isotropically very interesting group. Their distribution from the very high salt marsh experiencing fresh water ecosystem to the really low marsh, haline ecosystem (what is not really assumed) – in the case of C_{25} alkanes and mainly in the case of Bulk δ^{13}C values (see section 5.2.2). But Fig. 29 is clarifying the whole situation. The δ^{13}C values of C_{27} and C_{29} alkanes are proving the fresh water origin or distribution in the very high salt marsh area with high fresh water availability. The values of Lauroid angiosperms from Pecínov are really low: around -30‰ as well as in the case of *Eucalyptolaurus* from Hloubětín (what is not as haline locality as the Pecínov). The nearest values to them has *Pseudoasterophyllites* from Pecínov and it is assumed as very tolerant plant in case of environmental condition.

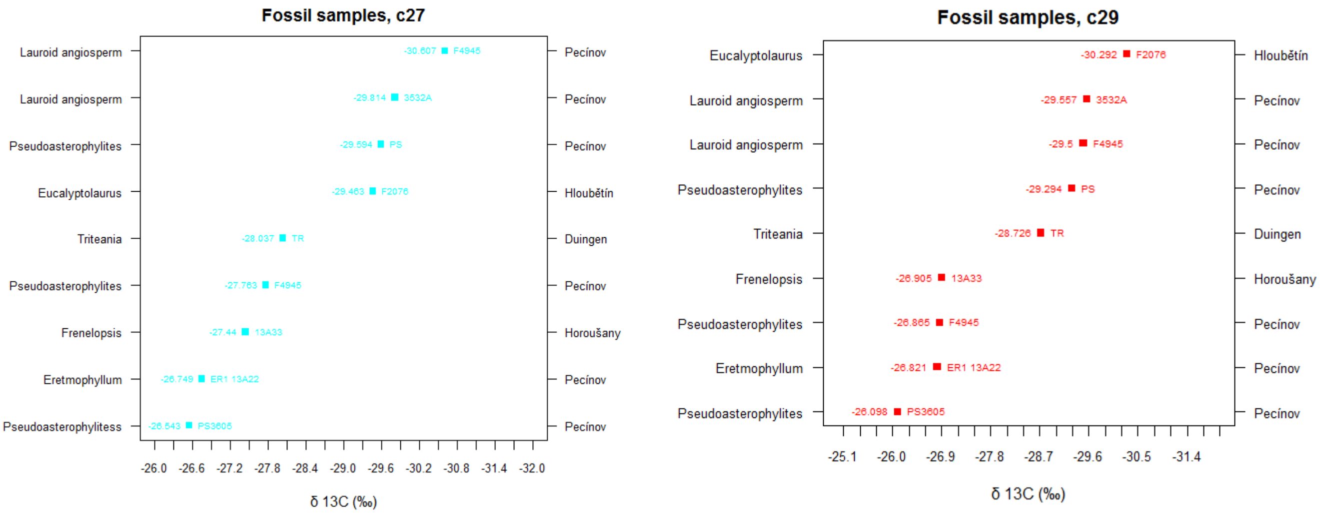


Figure 29: Plotted δ^{13}C n-alkanes (C_{27}, C_{29}) of fossil samples from all the localities – Pecínov, Hloubětín, Malá Chuchle, Horoušany and Duingen.

In the relative comparison of δ^{13}C values, *Pseudoasterophyllites* is interpreted as growing in the whole marsh, *Eretmophyllum* and *Frenelopsis* are interpreted as plants growing in low and middle marsh, *Tritaenia* is interpreted as growing in middle and high marsh and finally the Lauroid angiosperms are interpreted as growing in high marsh or environments with high fresh water availability. This interpretation is based mainly on the CSIA data, because as it was seen, in the case of Bulk data, there were many data which could lead to misinterpretation. Therefore the next section is dedicated to the problematic of differences between CSIA and Bulk analysis.

5.4 The isotopic shift between δ^{13}C Bulk and δ^{13}C CSIA

This problematic part of interpreting the data was well observed in the case of Lauroid angiosperms where the results of Bulk analysis were differently distributed than the results of CSIA. The shift between these two analyses is studied by Eley et al. (2016) on the recent samples. Therefore plot 30 shows how big the shift is between the Bulk and the CSIA values in the recent plants from the salt marshes (plots for other recent species are in the Appendix – Section 10).

To see the same shift in the fossil samples, the Bulk and the CSIA result data of fossil sample are plotted in plot 31. This plot can explain the very different data of Bulk and CSIA of Lauroid angiosperms. There is big divergence of the Bulk values and

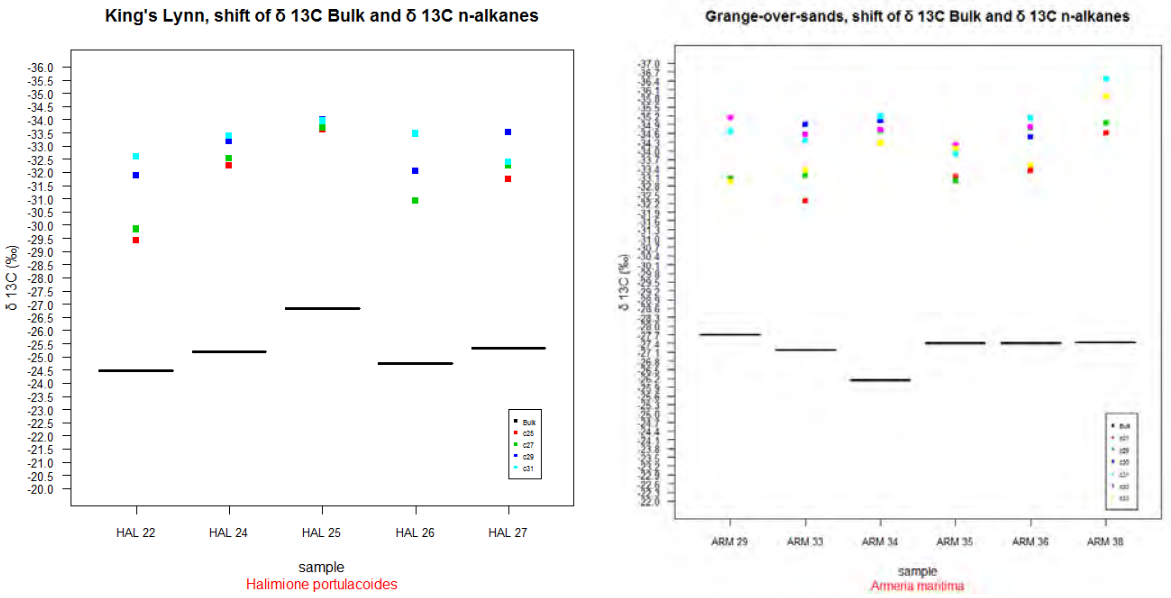


Figure 30: Plotted shifts of δ^{13}C n-alkanes against Bulk δ^{13}C of recent samples from all the salt marsh King's Lynn and Grange-over-sands. SAL – *Salicornia europaea*, ARM – *Armeria maritima*, GLA – *Glaux maritima*, HAL – *Halimione portulacoides*

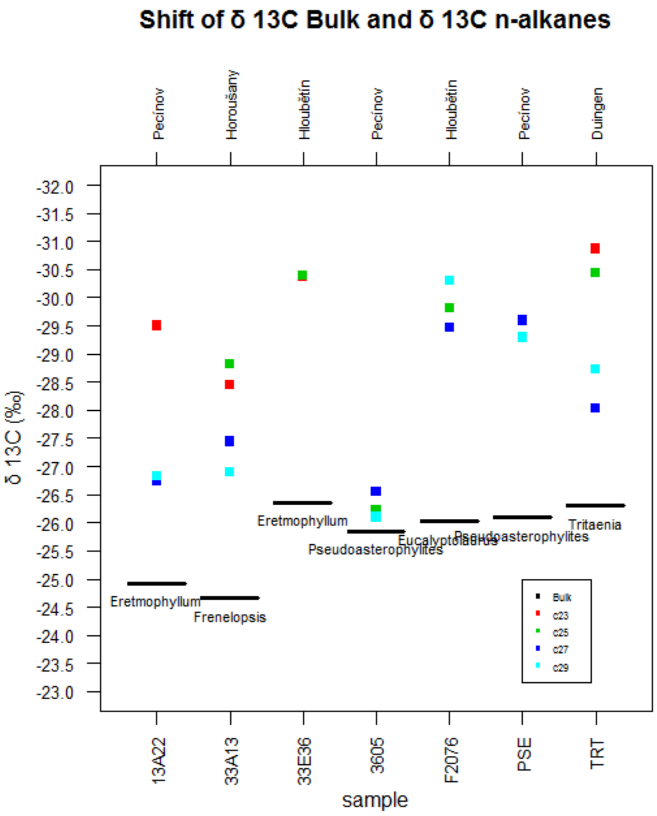


Figure 31: Plotted shifts of δ^{13}C n-alkanes against Bulk δ^{13}C of fossil samples from all localities.

the C_{27}, C_{29} values in the case of Lauroid angiosperm (*Eucalyptolaurus*) in comparison with the divergence of Bulk and the C_{27}, C_{29} values of other fossil species. Very close values are in the case of *Pseudoasterophyllites*, where is minimal isotopic shift. This is an evidence that in both analyses mainly the leaf waxes were measured, not any other organic matter (in the case of Bulk analysis). The result of this fact is that the fossil samples of *Pseudoasterophyllites* contain mainly the leaf waxes.

This plot is also showing which of the *n*-alkanes are the best for interpretation. The smallest divergence between the Bulk data and the CSIA data is in the case of C_{27}, C_{29}, which agrees very well with the sedimentological and palaeobotanical interpretations by Uličný et al. (1997a), Fatka and Kvaček (2006) and Kvaček (2016).

5.5 Comparing the modelled $\Delta_{LEAF-MAP}$ and $\Delta_{LEAF-LIPID}$ from the measured data

Comparison of the fossil data with recent measurements is not easy, because the values of $\delta^{13}C$ are driven by the atmospheric CO_2 concentration, by the precipitation and all this is connected with the temperature (Gröcke, 1998). One possibility how to compare fossil and recent samples is to calculate Δ_{LEAF} and compare it with modelled $\Delta_{LEAF-MAP}$ or with the recent Δ_{LEAF} values. Results of the calculation model $\Delta_{LEAF-MAP}$ and calculated Δ_{LEAF} are shown in the in Table 2, containing the data of the fossil samples. For comparison with recent data see tables in the Appendix (section 10).

In the table 2 it is possible to observe that some of the values are close to the modelled value, e.g., *Eucalyptolaurus* $\Delta_{LEAF-corrected}$ calculated from the $\Delta_{LEAF-alkane}$ corrected from the fractionation effect. Generally the calculated values of Δ are not the same as the modelled values of Δ, and this is probably caused by the very specific environment of salt marshes. There are probably more important and influencing factors which influence the $\delta^{13}C$ composition in the azonal ecosystem such as a salt marsh. Also the evidence of the closest values of *Eucalyptolaurus* support the interpretation of this plant – it was the less salt tolerant plant which were growing on the fresh water system. As close values can be considered the modelled and calculated Δ of *Frenelopsis*. These values fit better because the equation from conifers was used. The modelled Δ for conifers are calculated also for the recent localities and the values are lower than for the angiosperms. The fossil

					Calculated					Modelled	
name	C	average-δ^{13}C	locality	Species	$\delta^{13}C_{atm}$	$\Delta_{Leaf-alkane}$	ε_{lipid}	$\Delta_{Leaf-corrected}$	$\Delta_{Leaf-bulk}$	Δ Leaf-MAP - angiosperms	Δ Leaf-MAP - conifer
13E22	23	-29,50	Pecínov	Eretmophyllum	-5,69	24,54	-4,69	19,85	19,73		
13E22	27	-26,75	Pecínov	Eretmophyllum	-5,69	21,64	-1,87	19,77			
13E22	29	-26,82	Pecínov	Eretmophyllum	-5,69	21,72	-1,94	19,77			
33E36	23	-30,36	Hloubětín	Eretmophyllum	-6,99	24,10	-3,72	20,39	19,89		
33E36	25	-30,39	Hloubětín	Eretmophyllum	-6,99	24,13	-4,14	20,00			
33A13	23	-28,45	Horoušany	Frenelopsis (conif.)	-5,45	23,68	-3,89	19,79	19,70		20,19
33A13	25	-28,81	Horoušany	Frenelopsis (conif.)	-5,45	24,06	-4,26	19,80			
33A13	27	-27,44	Horoušany	Frenelopsis (conif.)	-5,45	22,62	-2,85	19,76			
33A13	29	-26,91	Horoušany	Frenelopsis (conif.)	-5,45	22,05	-2,30	19,75			
F2076	25	-29,81	Hloubětín	Eucalytolaurus	-6,68	23,84	-3,90	19,95	19,85	21,52	
F2076	27	-29,46	Hloubětín	Eucalytolaurus	-6,68	23,48	-1,80	21,67			
F2076	29	-30,29	Hloubětín	Eucalytolaurus	-6,68	24,35	-2,12	22,23			
3605	25	-26,23	Pecínov	Pseudoasterophylites	-6,53	20,24	-0,39	19,84	19,84		
3605	27	-26,54	Pecínov	Pseudoasterophylites	-6,53	20,56	-0,71	19,85			
3605	29	-26,10	Pecínov	Pseudoasterophylites	-6,53	20,09	-0,25	19,84			
PSE	27	-29,59	Pecínov	Pseudoasterophylites	-6,74	23,55	-3,60	19,95	19,86		
PSE	29	-29,29	Pecínov	Pseudoasterophylites	-6,74	23,23	-3,29	19,94			
TRT	23	-30,87	Duingen	Tritaenia	-6,94	24,68	-4,68	20,00	19,89		
TRT	25	-30,44	Duingen	Tritaenia	-6,94	24,23	-4,24	19,99			
TRT	27	-28,04	Duingen	Tritaenia	-6,94	21,70	-1,77	19,93			
TRT	29	-28,73	Duingen	Tritaenia	-6,94	22,43	-2,48	19,94			

Table 2: Table with calculated and modelled values of the fossil data. $\delta^{13}C_{atm}$ was calculated by (Arens et al., 2000) (equation 6), $\Delta_{LEAF-alkane}$, ϵ_{lipid}, $\Delta_{LEAF-corrected}$ and $\Delta_{LEAF-bulk}$ were calculated by Diefendorf et al. (2015) (equations 3, 4,5, 7).

values of Δ are in between these two modelled Δ. It might be because the fossil samples are also ginkgoalean plants and there is no model for this group of plants. The modelled Δ_{MAP} values (for both angiosperms and conifers) for the fossil environment are similar to the recent mangrove modelled values – see Table 3.1.3. This might be a strong argument for the mangrove-like environmental hypothesis.

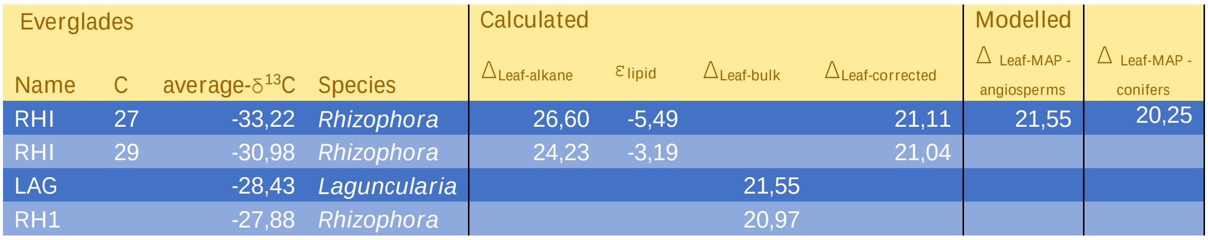
In the case of recent salt marsh values of Δ which can be seen in the Appendix (Section 10), the values of modelled $\Delta_{LEAF-MAP}$ compared with the calculated $\Delta_{LEAF-bulk}$ and $\Delta_{LEAF-corrected}$ are closer than in the case of the fossil samples, but they are not the same either. This fact supports the interpretation above – in the azonal ecosystem the δ^{13}C are driven by other factors than precipitation. Then if we compare the calculated fossil Δ with recent one, the values are oscillating in the same range. This is a good indicator that the fossil ecosystem was really the salt-marsh-like environment.

There is another idea which could be developed in a future research – is it possible to interpret the environment where the fossil plant was growing based on the similarity of the Δ values (the calculated recent and the calculated fossil)? If so, this algorithm could become a very powerful tool for palaeoenvironmental reconstructions.

5.6 Mangrove

All the previous analyses have been applied also to the samples from a recent mangrove. The question whether the palaeoenvironment was a mangrove-like or a salt marsh-like is very hard to answer; it may have been another ecosystem, which is not present in recent conditions. But the idea of a mangrove-like ecosystem could work as well as the salt marsh idea as far as the herbs were not found in the fossil record. The presence of herbs – concretely *Pseudoasterophyllites* – are the main reason why it is supposed that it was a salt marsh.

The results of measurements of the mangrove plants – *Rhizophora* and *Laguncularia* – are presented in Table 3. As it was mentioned above, the calculated Δ values are much closer to the modelled Δ values in the case of mangrove than in the case of the salt marsh or the fossil one (see Table 2). Generally they are higher than the salt marsh and the fossil values.



Everglades				Calculated				Modelled	
Name	C	average-δ^{13}C	Species	$\Delta_{Leaf\text{-}alkane}$	ε_{lipid}	$\Delta_{Leaf\text{-}bulk}$	$\Delta_{Leaf\text{-}corrected}$	Δ Leaf-MAP - angiosperms	Δ Leaf-MAP - conifers
RHI	27	-33,22	*Rhizophora*	26,60	-5,49		21,11	21,55	20,25
RHI	29	-30,98	*Rhizophora*	24,23	-3,19		21,04		
LAG		-28,43	*Laguncularia*			21,55			
RH1		-27,88	*Rhizophora*			20,97			

Table 3: Table with measured δ^{13}C Bulk, δ^{13}C n-alkanes, calculated and modelled values of the fossil data. $\delta^{13}C_{atm}$ was calculated by (Arens et al., 2000) (equation 6), $\Delta_{LEAF-alkane}$, ϵ_{lipid}, $\Delta_{LEAF-corrected}$ and $\Delta_{LEAF-bulk}$ were calculated by Diefendorf et al. (2015) (equations 3, 4, 5, 7).

The mangroves are not totally out of the "game", because there can be another interpretation of the Lauroid angiosperms which is based on their δ^{13}C values. As it was shown above, the bulk data are not very informative in the case of lauroid angiosperms and the salt marsh idea. But in the case of trusting them, the mangrove-like environment is a good interpretation. The CSIA data are supporting the more water available conditions and in mangroves the values of δ^{13}C are very low, because the trees are growing directly in the water. Then the Bulk data reflect the isotopic composition of all the carbon in the sample – other parts of the leaf can have higher values because of the sediment character (there it is necessary to presuppose in situ preservation) and the leaf waxes values are very low, because the plant itself is growing in the water, so the water availability is high.

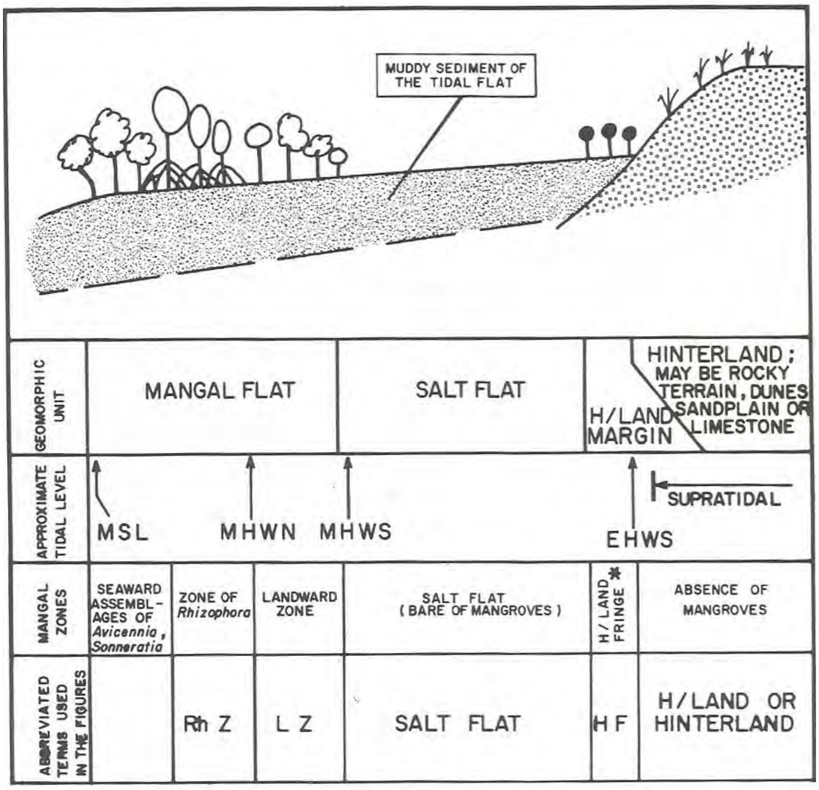


Figure 32: Schematic profile of tidal flat illustrating the mangrove zonation. MSL – mean sea level, MHWN – mean high water at neap tide, MHWS – mean high water at spring tide, EHWS – extreme high water at spring tide (Semeniuk, 1983).

Also the cuticular analysis can be interpreted as supporting this ideal – the adaxial cuticle is thicker and has no stomata and the abaxial is very thin with stomata. The adaxial surface of the leaf is protected against the sun irradiation which is higher thanks to the higher albedo of the water. This idea leads to a totally different alternative model based on the recent mangrove distribution (see Fig. 32), where the Lauroid angiosperms would grow as the mangrove-like plants in the zone of today's *Rhizophora* zone – mangal flat, then *Frenelopsis* is growing in the landward zone, *Pseudoasterophyllites* is growing in the salt flat zone and ginkgoalean plants are in the land margin.

There is no evidence that this hypothesis is correct, even in Unit 3 in the Pecínov quarry is caped by rootlet zone. For supporting this hypothesis, it is recommended to analyse the mangrove vegetation by the same methods as were applied to the salt marshes and improve the algorithm for possibility to compare fossil data with the recent ones as was tested by Diefendorf et al. (2015). But it is possible that it could be rejected based just on the morphological structures of the leaves. Nevertheless, it is important to keep in mind that the climatic conditions were different than today's, and therefore the mangrove-like hypothesis is suggested as an alternative hypothesis.

6 Discussion

Recent salt marsh δ^{13}C bulk data Because of the mosaic structure of the salt marsh (see Fig. 21), there is high possibility that even in one location the microhabitats differ in each 10 cm. This phenomenon could cause the wide range of δ^{13}C values in one locality. The samples are showing that the plants are growing in conditions which are suitable for their life, therefore in one location, we have a wide range of values. They are not growing in 1 cm area, therefore, during the collection there were probably different conditions in a very small area (less than square 3×3 m). This shows us how mosaic the salt marsh is.

Plantago can grow in the low water stressed area and next to this area one can find *Salicornia* and *Glaux* in the high water stress conditions. These two microhabitats are less than 3 m apart. And it is more than clear that *Plantago* is occupying the area with water availability and therefore the salt tolerant species are forced to grow in "worse" conditions, where *Plantago* couldn't survive. This phenomenon is shown in location 37, 35 (*Plantago* and *Armeria* are the less salt-tolerant and occupy more humid soil), 33 (*Suaeda* is growing in more humid soil than the others species), etc.

Another possible interpretation of this wide range values in one location could be the fractionation. If *Plantago* is more salt tolerant, then the stress wouldn't be as high as in the case of *Salicornia* and *Glaux* (in the case of location 37). This interpretation is not very likely, because *Salicornia* is well known as a salt-tolerant plant, more than *Plantago*, therefore the interpretation above (mosaic distribution in small range) is more likely.

Fossil δ^{13}C bulk data The problem (Fig. 22) with the results of bulk δ^{13}C values is based on the fossil material. Especially in the fossil record, the sample is influenced by many factors and the measured data are from all organic matter presented in the measured sample.

If we trust these data, we have to start to think about the environment, where the Lauroid angiosperms could live. It is a well known fact that in the Early Cretaceous the Lauroid angiosperms underwent a big explosion, trying to invade many environments and free niches (Friis et al., 2011). Therefore there might be hypothesis that these 'halophytic' Lauroid angiosperms were really growing in the salt marsh conditions trying to invade this environment. Today, there are no trees growing in the salt marsh, but during this

Lauroid explosion, it could be possible that they tried all the possible environments to survive and spread.

Other problems with interpreting the fossil data are discussed below.

Recent salt marsh δ^{13}C *n*-alkane data In the δ^{13}C *n*-alkane data, we encounter the same problem occurs as in the case of the bulk data. There is no gradient and the mosaic distribution of vegetation is the only theory which can explain these data. As a topic for future research, it could be very useful to measure the salinity and other parameters of the soil during the process of sampling. Then it would be possible to determine the factors that have a significant influence on the distribution of plants.

In the case of recent samples there is more than one problematic part of the interpretation, because by Eley et al. (2016) there is variation of the δ^{13}C composition during time. This variation is not caused (in the case of C3 plants) by seasonal patterns or growth strategy. A potential explanation is the changes in plant physiology and biochemistry in response to the environmental stresses over the growth season – salinity, nitrogen limitation, root anoxia, water-logging, changes in redox potential, concentration of toxic chemicals. These facts support the hypothesis that it is possible to use δ^{13}C for palaeoreconstructions, but it is important to keep in mind how many factors (listed above) are causing the stressful environment. It is not possible to say directly which factor influenced the fossil plant and after combining this with the mosaic distribution of the plants in the salt marsh, it is very hard to reconstruct the palaeoenvironment in detail.

Time is another problematic dimension. The δ^{13}C is changing in time scale which it is not possible to follow in the fossil record.

Nevertheless, this thesis is supporting the previous ideas of distribution of the fossil samples ((Fatka and Kvaček, 2006), (Kvaček, 2016)) which are based on the sedimentological and palaeobotanical observations. The isotopic data bring another proxy (δ^{13}C bulk and CSIA) which is specifying the environmental conditions.

Fossil δ^{13}C *n*-alkane data The problematic part of this proxy method is the quantity. The CSIA is a very powerful method for the recent samples but the application of this method on the fossil samples has its limitations. Because it is necessary to extract the leaf waxes, the amount of the sample has to be higher than for the Bulk analysis. It really depends on the thickness of the cuticle and on the content of leaf waxes. Plants with small content of leaf waxes are very difficult to analyse as long as there is enough material for the separation. There is no given amount of the plant for the separation just because each species has different amount of the leaf wax, therefore this part of processing samples is very intuitive and based mainly on earlier experiences.

There is also another variable and it is the solvent which is used. Hexane was used, but it is possible to use another non-polar solvent which can be more efficient. Because of low concentration of the fossil samples, there is high risk of contamination.

In the interpretation of fossil δ^{13}C n-alkane data, it is very important to have some essential information about the locality, the species and their supposed habitat. The results cannot be compared directly with analogous recent ecosystems mainly because of the different atmospheric conditions. In this case, it is not possible also because the fossil species are extinct and there are no living relatives living in the same conditions as it was in the Cretaceous. Another risk factor is that here are compared trees, shrubs with herbs and also the number of samples from one locality is not very high. Therefore, in this thesis there is no effort to compare the samples with the recent one (or the fossils one from different localities) based just on the absolute isotopic composition. The main importance is on the trends, gradients or environmental factors which could influence the δ^{13}C composition.

The modelling of $\Delta_{LEAF-MAP}$ and $\Delta_{LEAF-LIPID}$ from the measured data The method which was used is very new and it was applied by Diefendorf et al. (2015) on the Palaeogene samples. The main difficulty of applying this method on the Cretaceous samples was to find or calculate environmental variables which are used in the formulas from Diefendorf et al. (2015). The atmospheric δ^{13}C was calculated from the δ^{13}C Bulk data using the formula by Arens et al. (2000). This formula is calculating the $\delta^{13}\mathrm{C}_{air}$, what is not the same as the atmospheric δ^{13}C. It is the composition of the air outside the plant, therefore the calculated atmospheric δ^{13}C is very specific for the locality and it varies within species. The formula by Diefendorf et al. (2015) is designed for the atmospheric δ^{13}C, therefore it can be incorrect to use the $\delta^{13}\mathrm{C}_{air}$. For the future calculations of atmospheric δ^{13}C there should be used the data from benthic foraminiferas.

Another problem can occur in the calculation of the model $\Delta_{LEAF-MAP}$. Diefendorf et al. (2015) use two formulas – one is for modelling angiosperms $\Delta_{LEAF-MAP}$ and the second is for conifers $\Delta_{LEAF-MAP}$. But the fossil samples are also represented by Ginkgoales and for this group of plants, none of these equations might be applicable. It is a question if the calculation of models is right in the case of ginkgoalean plants. Finally, it was a test of this method on the Cretaceous data and the results could be influenced by many errors and mistakes, therefore it is necessary to look on the results very carefully.

Mangrove hypothesis As it was supposed above, the hypothesis that the palaeoenvironment was mangrove-like could be supported and also not supported by different facts. The supporting factor is temperature. The mean annual temperature in the Bohemian Cretaceous basin in Cenomanian was $17,3$℃to $20,2$℃across all localities (the warmest locality is Malá Chuchle) (Herman et al., 2002). The palaeoaltitude of the the Bohemian Cretaceous basin in Cenomanian was 40 °N (Herman et al., 2002) and even the today's limitation of mangroves is 28 °N and the limiting temperature is 20 ℃(Packham and Willis, 1997; Ranwell, 1972), there is still probability that in these conditions were mangrove-like vegetation. The limits are on the border of the interval, therefore some intermediate environment, something between salt marsh and the mangroves could occur. The modelled and the calculated Δ values from mangrove and fossil samples are very close values, therefore this is a strong argument supporting the mangrove-like environment. To solve this hypothesis, it would be recommended to analyse the mangrove vegetation by the same methods as were applied to the salt marshes.

7 Conclusions

In this thesis, recent salt marsh plants were measured by Bulk analysis and CSIA to model the salt marsh gradient. The environmental conditions were defined for each of the recent samples based on the mosaic vegetation model by Othman (1980). This model was used for the interpretation of three fossil cases – the Bohemian Cretaceous Basin (Pecínov, Horoušany, Hloubětín and Malá Chuchle), the Lower Saxony Basin (Duingen) and the Jurassic of Yorkshire (Scalby Mills). All the fossil samples were measured by both methods – Bulk analysis and CSIA, but only some of the measurements produced usable data. All the data were processed and plotted to find relationships between the samples. The Bulk analysis showed very disputable results which were clarified by the CSIA. The main difference in the Bulk and CSIA values were documented in Lauroid angiosperms. The Bulk data were distributed in a wide range of values and indicated haline environment as well as fresh water environment. Based on the CSIA data, Lauroid angiosperm were interpreted as fresh water plants or the very high salt marsh zone ecosystem with high water availability. An alternative hypothesis about the mangrove-like distribution was suggested based on the isotopic values of lauroid angiosperms.

Frenelopsis and *Eretmophyllum* were interpreted as salt tolerant plants with habitat close to recent *Halimione portulacoides* – low water availability, stable salinity. The distribution would be around the tidal creeks in the middle and high marsh. The isotopic values of *Pseudoasterophyllites* were similar in both types of analysis (Bulk and CSIA) and this indicated the purity of the measured material, namely, it pointed to the absence of organic carbon compounds other than the leaf waxes in the measured samples.

Pseudoasteriphyllites is interpreted as a herb growing in the pioneer zone of the salt marsh as well as in all other microhabitats, thanks to its ability to buffer extreme conditions. *Pseudoaterophillites* is compared to recent *Salicornia* which grows in microhabitats with high water availability but very variable salinity. It can grow in very saline conditions as well as dry conditions and also in humid environments with low salinity. This is caused by *Salicornia*'s good ability to buffer the extreme conditions.

Tritaenia as a sample from the Saxonian Basin in Germany was interpreted as a slightly salt tolerant plant which grew in the highest salt marsh zones or in fresh water ecosystem. Its isotopic values were close to the values of Lauroid angisosperms and therefore the interpretation of the environment is similar. Because of the absence of any other samples from this locality, it was not possible to model specific environmental conditions and the interpretation is derived from the relative comparison with other fossil isotopic data. But the sedimentological and palaeobotanical interpretations of *Tritaenia* were supported by these isotopic data.

Ginkgo huttonii provided only the Bulk data. No signal was recorded in the CSIA analysis because of the small amount of material. The Bulk data were interpreted as the fresh water plant with a slight salt tolerance. The values were close to the isotopic values of Lauroid angiosperms. From the Jurassic locality in GB we have no more material to compare, therefore the interpretation was based on the relative relationship with other isotopic data of fossil samples. The sedimentological and palaeobotanical data could be supported by isotopic data.

The differences between the values of the Bulk analysis and CSIA were plotted. The results show that the Bulk data are not as informative as the leaf waxes data, and therefore it is recommended to use CSIA in palaeoenvironment reconstruction to obtain more precise information about the environment.

A new promising method to compare fossil data with recent ones was used. A parameter Δ was calculated form the measured data and compared to another parameter Δ_{MAP}, which was modelled from mean annual precipitation data. The comparison showed that only in the case of the recent mangrove, the modelled values were similar to the values calculated from the measured data. In the case of recent and fossil plants from salt marsh ecosystems, the values of the two parameters were slightly different. It is disputable whether this method can be used for the salt marsh ecosystem because its azonality. Therefore, the CSIA method – tested here on European Mesozoic plants for the first time – will need to be subjected to future research designed to improve this algorithm, which could prove to be a very powerful tool in palaeorecontructions.

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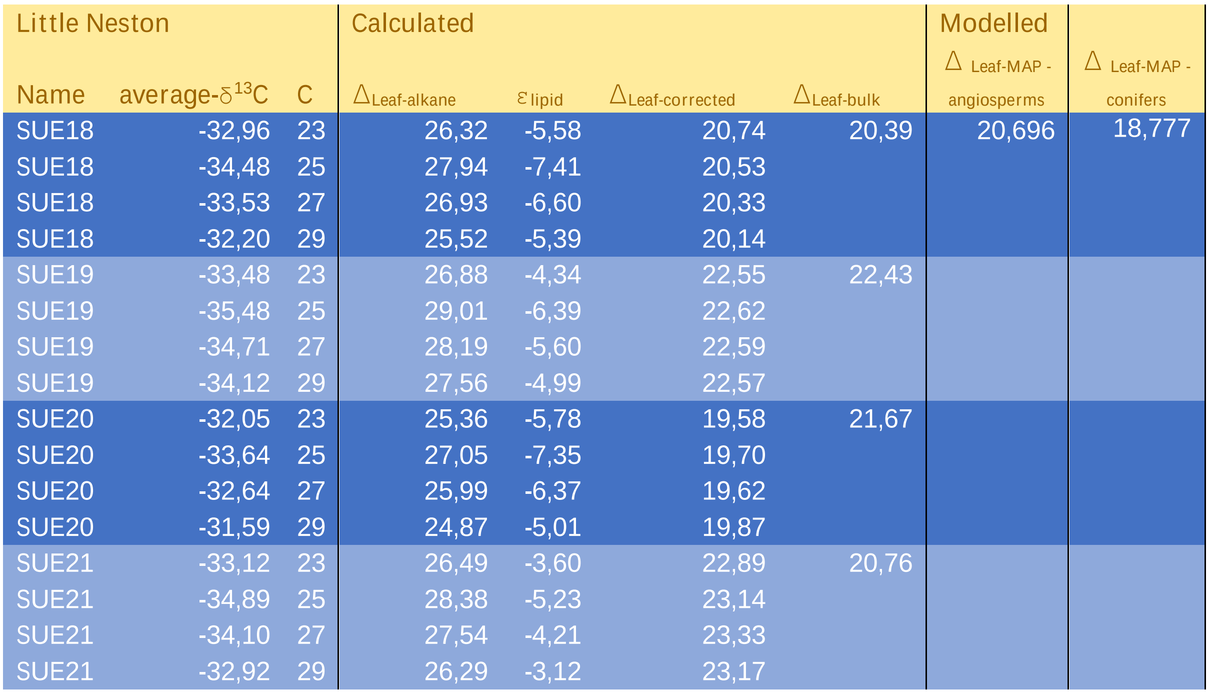
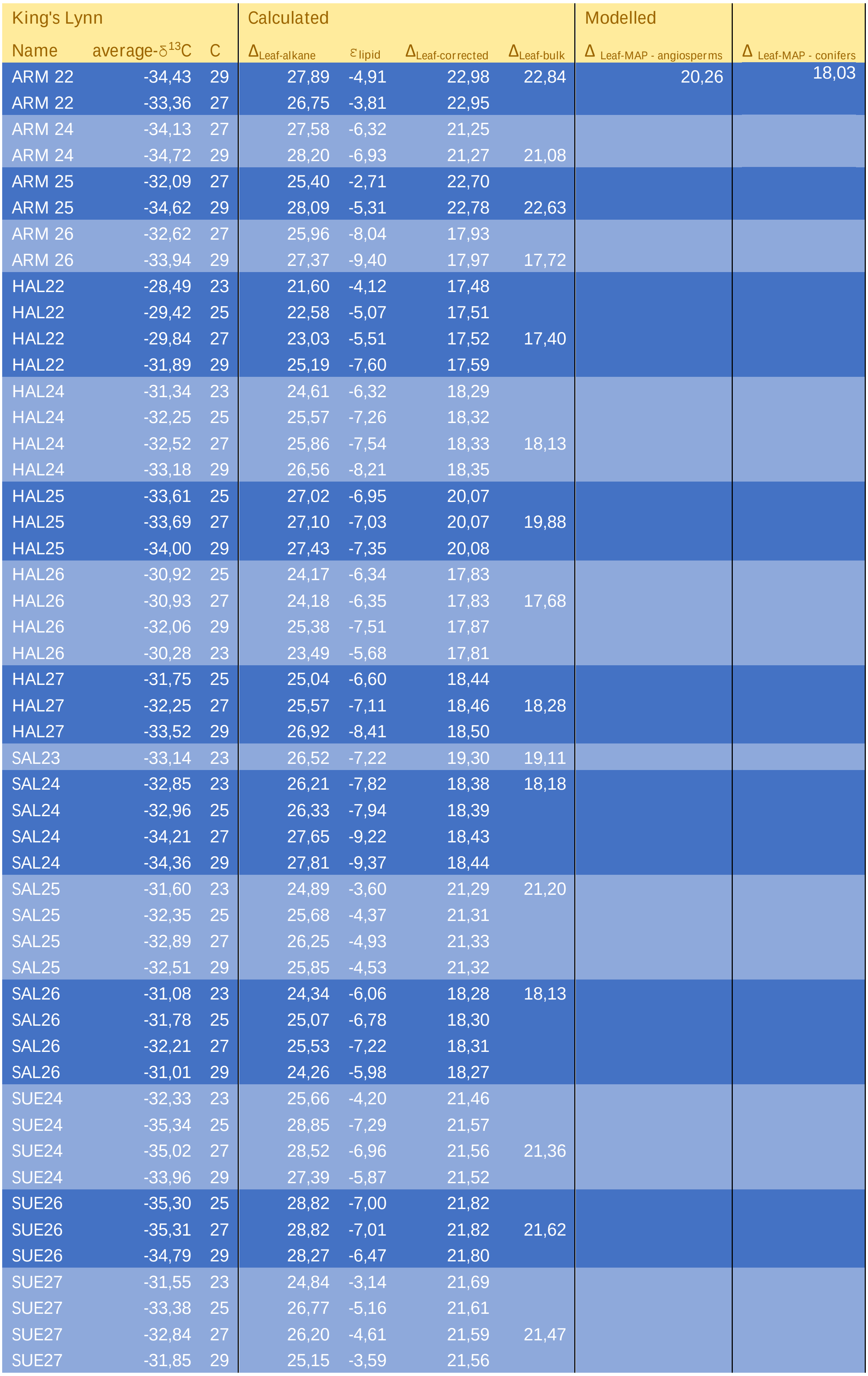
10 Appendix



Little Neston			Calculated				Modelled	
Name	average-δ^{13}C	C	$\Delta_{\text{Leaf-alkane}}$	$\varepsilon_{\text{lipid}}$	$\Delta_{\text{Leaf-corrected}}$	$\Delta_{\text{Leaf-bulk}}$	Δ Leaf-MAP - angiosperms	Δ Leaf-MAP - conifers
SUE18	-32,96	23	26,32	-5,58	20,74	20,39	20,696	18,777
SUE18	-34,48	25	27,94	-7,41	20,53			
SUE18	-33,53	27	26,93	-6,60	20,33			
SUE18	-32,20	29	25,52	-5,39	20,14			
SUE19	-33,48	23	26,88	-4,34	22,55	22,43		
SUE19	-35,48	25	29,01	-6,39	22,62			
SUE19	-34,71	27	28,19	-5,60	22,59			
SUE19	-34,12	29	27,56	-4,99	22,57			
SUE20	-32,05	23	25,36	-5,78	19,58	21,67		
SUE20	-33,64	25	27,05	-7,35	19,70			
SUE20	-32,64	27	25,99	-6,37	19,62			
SUE20	-31,59	29	24,87	-5,01	19,87			
SUE21	-33,12	23	26,49	-3,60	22,89	20,76		
SUE21	-34,89	25	28,38	-5,23	23,14			
SUE21	-34,10	27	27,54	-4,21	23,33			
SUE21	-32,92	29	26,29	-3,12	23,17			

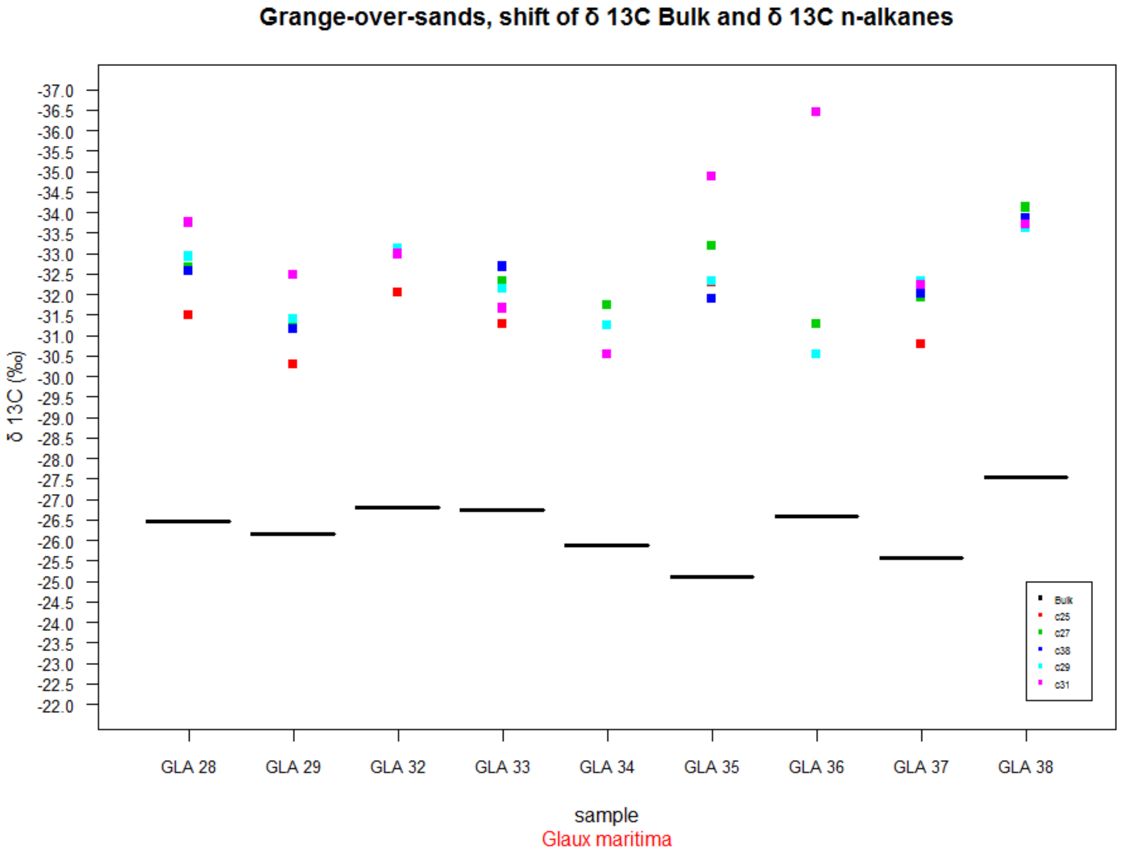


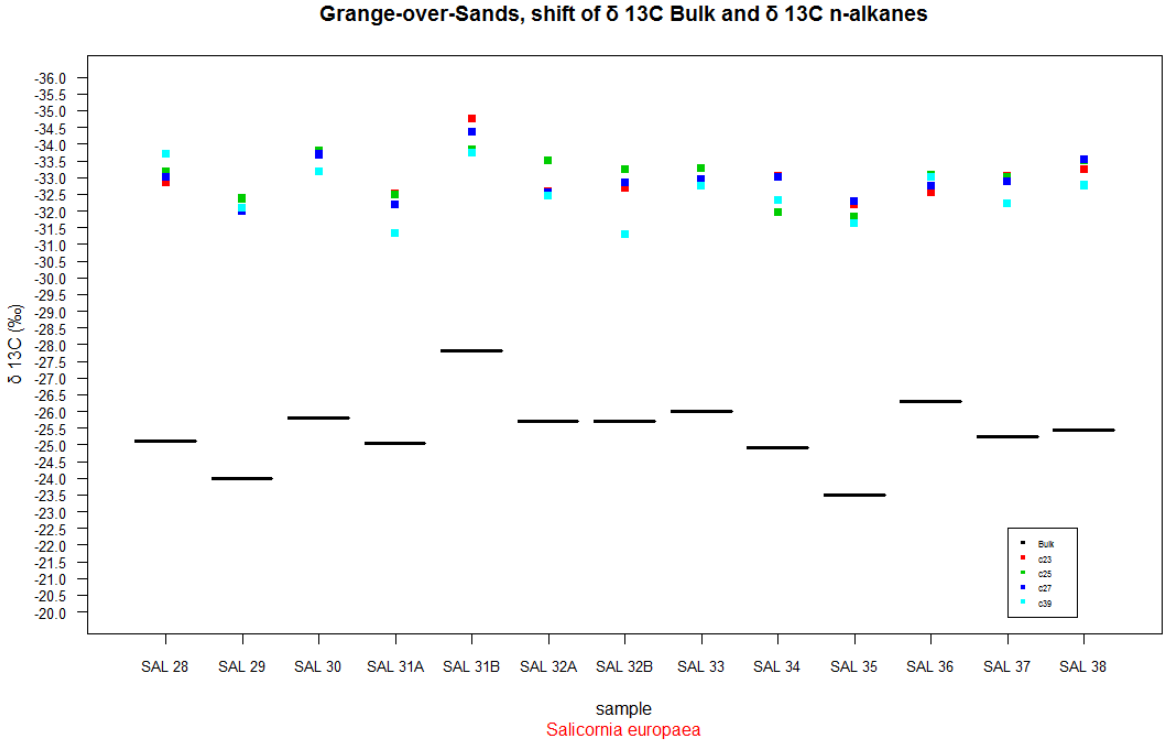
King's Lynn			Calculated				Modelled	
Name	average-δ^{13}C	C	$\Delta_{\text{Leaf-alkane}}$	$\varepsilon_{\text{lipid}}$	$\Delta_{\text{Leaf-corrected}}$	$\Delta_{\text{Leaf-bulk}}$	Δ Leaf-MAP - angiosperms	Δ Leaf-MAP - conifers
ARM 22	-34,43	29	27,89	-4,91	22,98	22,84	20,26	18,03
ARM 22	-33,36	27	26,75	-3,81	22,95			
ARM 24	-34,13	27	27,58	-6,32	21,25			
ARM 24	-34,72	29	28,20	-6,93	21,27	21,08		
ARM 25	-32,09	27	25,40	-2,71	22,70			
ARM 25	-34,62	29	28,09	-5,31	22,78	22,63		
ARM 26	-32,62	27	25,96	-8,04	17,93			
ARM 26	-33,94	29	27,37	-9,40	17,97	17,72		
HAL22	-28,49	23	21,60	-4,12	17,48			
HAL22	-29,42	25	22,58	-5,07	17,51			
HAL22	-29,84	27	23,03	-5,51	17,52	17,40		
HAL22	-31,89	29	25,19	-7,60	17,59			
HAL24	-31,34	23	24,61	-6,32	18,29			
HAL24	-32,25	25	25,57	-7,26	18,32			
HAL24	-32,52	27	25,86	-7,54	18,33	18,13		
HAL24	-33,18	29	26,56	-8,21	18,35			
HAL25	-33,61	25	27,02	-6,95	20,07			
HAL25	-33,69	27	27,10	-7,03	20,07	19,88		
HAL25	-34,00	29	27,43	-7,35	20,08			
HAL26	-30,92	25	24,17	-6,34	17,83			
HAL26	-30,93	27	24,18	-6,35	17,83	17,68		
HAL26	-32,06	29	25,38	-7,51	17,87			
HAL26	-30,28	23	23,49	-5,68	17,81			
HAL27	-31,75	25	25,04	-6,60	18,44			
HAL27	-32,25	27	25,57	-7,11	18,46	18,28		
HAL27	-33,52	29	26,92	-8,41	18,50			
SAL23	-33,14	23	26,52	-7,22	19,30	19,11		
SAL24	-32,85	23	26,21	-7,82	18,38	18,18		
SAL24	-32,96	25	26,33	-7,94	18,39			
SAL24	-34,21	27	27,65	-9,22	18,43			
SAL24	-34,36	29	27,81	-9,37	18,44			
SAL25	-31,60	23	24,89	-3,60	21,29	21,20		
SAL25	-32,35	25	25,68	-4,37	21,31			
SAL25	-32,89	27	26,25	-4,93	21,33			
SAL25	-32,51	29	25,85	-4,53	21,32			
SAL26	-31,08	23	24,34	-6,06	18,28	18,13		
SAL26	-31,78	25	25,07	-6,78	18,30			
SAL26	-32,21	27	25,53	-7,22	18,31			
SAL26	-31,01	29	24,26	-5,98	18,27			
SUE24	-32,33	23	25,66	-4,20	21,46			
SUE24	-35,34	25	28,85	-7,29	21,57			
SUE24	-35,02	27	28,52	-6,96	21,56	21,36		
SUE24	-33,96	29	27,39	-5,87	21,52			
SUE26	-35,30	25	28,82	-7,00	21,82			
SUE26	-35,31	27	28,82	-7,01	21,82	21,62		
SUE26	-34,79	29	28,27	-6,47	21,80			
SUE27	-31,55	23	24,84	-3,14	21,69			
SUE27	-33,38	25	26,77	-5,16	21,61			
SUE27	-32,84	27	26,20	-4,61	21,59	21,47		
SUE27	-31,85	29	25,15	-3,59	21,56			

Grange-over-sands			Calculated				Modelled	
Name	average-δ^{13}C	C	$\Delta_{Leaf\text{-}alkane}$	ε_{lipid}	$\Delta_{Leaf\text{-}corrected}$	$\Delta_{Leaf\text{-}bulk}$	$\Delta_{Leaf\text{-}MAP\ -\ angiosperms}$	$\Delta_{Leaf\text{-}MAP\ -\ conifers}$
ARM 29	-33,08	29	26,45	-7,72	18,73	20,76	20,69	18,78
ARM 33	-33,16	29	26,53	-7,60	18,94	20,21		
ARM 33	-32,28	27	25,61	-7,36	18,25			
ARM 34	-34,69	29	28,17	-5,73	22,44	19,15		
ARM 35	-32,97	29	26,34	-8,78	17,56	20,46		
ARM 35	-33,09	27	26,46	-6,07	20,39			
ARM 36	-33,31	27	26,70	-5,85	20,85	20,47		
ARM 36	-34,79	29	28,28	-5,54	22,74			
ARM 38	-34,61	27	28,08	-5,27	22,82	20,52		
ARM 38	-34,96	29	28,45	-6,16	22,29			
GLA 28	-32,66	27	26,01	-6,37	19,64	19,48		
GLA 28	-32,93	29	26,30	-6,64	19,65			
GLA 28	-31,49	25	24,77	-5,17	19,60			
GLA 29	-31,31	27	24,58	-5,29	19,28	19,15		
GLA 29	-31,41	29	24,68	-5,40	19,29			
GLA 29	-30,29	25	23,50	-4,25	19,25			
GLA 32	-32,04	25	25,35	-5,37	19,98	19,85		
GLA 32	-33,14	27	26,52	-6,50	20,02			
GLA 32	-33,12	29	26,50	-6,48	20,02			
GLA 33	-32,31	27	25,64	-5,74	19,91	19,76		
GLA 33	-32,15	29	25,47	-5,57	19,90			
GLA 33	-31,29	25	24,56	-4,69	19,87			
GLA 34	-31,74	27	25,03	-6,03	19,01	18,86		
GLA 34	-31,25	29	24,52	-5,53	18,99			
GLA 35	-33,20	27	26,58	-8,30	18,28	18,06		
GLA 35	-32,13	29	25,45	-7,21	18,24			
GLA 35	-32,32	25	25,64	-7,40	18,25			
GLA 36	-31,29	27	24,56	-4,83	19,73	19,61		
GLA 36	-30,54	29	23,77	-4,06	19,71			
GLA 37	-31,93	27	25,23	-6,53	18,70	18,53		
GLA 37	-32,33	29	25,66	-6,95	18,71			
GLA 37	-30,80	25	24,04	-5,37	18,66			
GLA 38	-34,13	27	27,57	-6,78	20,78	20,60		
GLA 38	-33,62	29	27,03	-6,26	20,77			
HAL38	-32,15	25	25,47	-6,04	19,43	19,28		
HAL38	-32,66	27	26,01	-6,56	19,45			
HAL38	-33,52	29	26,92	-7,44	19,48			
PLA31	-35,43	27	28,96	-7,23	21,73	21,52		
PLA31	-37,72	29	31,41	-9,58	21,82			
PLA32	-33,42	27	26,82	-5,23	21,59	21,45		
PLA32	-33,91	29	27,34	-5,73	21,61			
PLA34	-34,80	27	28,28	-7,54	20,74	20,53		
PLA34	-34,95	29	28,44	-7,69	20,75			
PLA35	-26,64	23	19,66	1,29	20,96	20,99		
PLA35	-26,31	25	19,32	1,64	20,95			
PLA35	-28,30	27	21,40	-0,41	20,99			
PLA35	-34,41	29	27,87	-6,70	21,17			
PLA36	-33,21	25	26,59	-6,28	20,31	20,14		
PLA36	-35,51	27	29,04	-8,64	20,39			
PLA36	-35,49	29	29,01	-8,62	20,39			

PLA37	-37,01	27	30,64	-8,09	22,56	22,31		
PLA37	-37,20	29	30,85	-8,29	22,56			
PLA38	-31,85	23	25,15	-4,02	21,14	21,04		
PLA38	-32,35	25	25,68	-4,53	21,15			
PLA38	-35,80	27	29,35	-8,08	21,27			
PLA38	-34,46	29	27,92	-6,70	21,22			
SAL28	-32,84	23	26,20	-7,92	18,28	18,07		
SAL28	-33,18	25	26,56	-8,27	18,29			
SAL28	-33,00	27	26,36	-8,08	18,29			
SAL28	-33,71	29	27,12	-8,81	18,31			
SAL29	-32,01	23	25,32	-8,20	17,12	16,91		
SAL29	-32,36	25	25,70	-8,57	17,13			
SAL29	-32,00	27	25,31	-8,20	17,12			
SAL29	-32,10	29	25,41	-8,29	17,12			
SAL30	-33,67	23	27,08	-8,08	19,00	18,78		
SAL30	-33,80	25	27,22	-8,22	19,00			
SAL30	-33,68	27	27,10	-8,10	19,00			
SAL30	-33,17	29	26,55	-7,57	18,98			
SAL31A	-32,50	23	25,83	-7,65	18,19	17,99		
SAL31A	-32,49	25	25,83	-7,64	18,19			
SAL31A	-32,19	27	25,51	-7,33	18,18			
SAL31A	-31,34	29	24,61	-6,46	18,15			
SAL31B	-34,76	23	28,24	-7,13	21,11	20,91		
SAL31B	-33,84	25	27,26	-6,19	21,08			
SAL31B	-34,37	27	27,82	-6,72	21,10			
SAL31B	-33,74	29	27,15	-6,08	21,08			
SAL32A	-32,58	23	25,92	-7,05	18,87	18,69		
SAL32A	-33,51	25	26,91	-8,01	18,90			
SAL32A	-32,55	27	25,90	-7,03	18,87			
SAL32A	-32,44	29	25,78	-6,91	18,87			
SAL32B	-32,69	23	26,04	-7,17	18,87	18,68		
SAL32B	-33,23	25	26,61	-7,72	18,89			
SAL32B	-32,84	27	26,20	-7,32	18,88			
SAL32B	-31,28	29	24,55	-5,73	18,83			
SAL33	-32,79	23	26,14	-6,97	19,18	19,00		
SAL33	-33,26	25	26,65	-7,46	19,19			
SAL33	-32,93	27	26,30	-7,12	19,18			
SAL33	-32,73	29	26,09	-6,91	19,18			
SAL34	-33,02	23	26,40	-8,33	18,07	17,85		
SAL34	-31,94	25	25,25	-7,22	18,03			
SAL34	-33,02	27	26,39	-8,32	18,07			
SAL34	-32,32	29	25,65	-7,61	18,04			
SAL35	-32,19	23	25,51	-8,89	16,62	16,39		
SAL35	-31,81	25	25,11	-8,51	16,60			
SAL35	-32,29	27	25,62	-9,00	16,62			
SAL35	-31,63	29	24,92	-8,33	16,60			
SAL36	-32,53	23	25,88	-6,39	19,48	19,32		
SAL36	-33,08	25	26,46	-6,95	19,50			
SAL36	-32,75	27	26,11	-6,62	19,49			
SAL36	-32,99	29	26,36	-6,87	19,50			
SAL37	-32,87	27	26,23	-7,82	18,42	18,21		
SAL37	-32,20	29	25,53	-7,13	18,39			

SAL37	-33,03	23	26,40	-7,98	18,42			
SAL37	-33,00	25	26,37	-7,95	18,42			
SAL38	-33,24	23	26,62	-8,00	18,62	18,41		
SAL38	-33,51	25	26,91	-8,28	18,63			
SAL38	-33,52	27	26,93	-8,30	18,63			
SAL38	-32,76	29	26,12	-7,51	18,60			
SUE29	-33,90	27	27,32	-4,17	23,16	23,04		
SUE29	-33,96	29	27,39	-4,23	23,16			
SUE30	-33,03	23	26,41	-3,21	23,20	23,11		
SUE30	-35,28	25	28,80	-5,53	23,27			
SUE30	-35,36	27	28,88	-5,61	23,27			
SUE30	-35,15	29	28,65	-5,39	23,26			
SUE31	-31,82	23	25,12	-4,71	20,40	20,28		
SUE31	-33,63	25	27,04	-6,58	20,46			
SUE31	-34,18	27	27,62	-7,14	20,48			
SUE31	-33,61	29	27,02	-6,56	20,46			
SUE33	-34,62	25	28,10	-5,14	22,96	22,81		
SUE33	-34,62	27	28,09	-5,14	22,96			
SUE33	-33,68	29	27,09	-4,17	22,93			
SUE37	-33,73	23	27,14	-6,68	20,47	19,64		
SUE37	-34,33	25	27,79	-7,93	19,86			
SUE37	-34,30	27	27,75	-7,90	19,86			
SUE37	-33,92	29	27,35	-7,51	19,84			


Grange-over-sands, shift of δ 13C Bulk and δ 13C n-alkanes
δ 13C (‰)
-37.0
-36.5
-36.0
-35.5
-35.0
-34.5
-34.0
-33.5
-33.0
-32.5
-32.0
-31.5
-31.0
-30.5
-30.0
-29.5
-29.0
-28.5
-28.0
-27.5
-27.0
-26.5
-26.0
-25.5
-25.0
-24.5
-24.0
-23.5
-23.0
-22.5
-22.0
GLA 28
GLA 29
GLA 32
GLA 33
GLA 34
GLA 35
GLA 36
GLA 37
GLA 38
sample
Glaux maritima
Bulk
c25
c27
c38
c29
c31


Grange-over-Sands, shift of δ 13C Bulk and δ 13C n-alkanes
δ 13C (‰)
-36.0
-35.5
-35.0
-34.5
-34.0
-33.5
-33.0
-32.5
-32.0
-31.5
-31.0
-30.5
-30.0
-29.5
-29.0
-28.5
-28.0
-27.5
-27.0
-26.5
-26.0
-25.5
-25.0
-24.5
-24.0
-23.5
-23.0
-22.5
-22.0
-21.5
-21.0
-20.5
-20.0
SAL 28
SAL 29
SAL 30
SAL 31A
SAL 31B
SAL 32A
SAL 32B
SAL 33
SAL 34
SAL 35
SAL 36
SAL 37
SAL 38
sample
Salicornia europaea
Bulk
c23
c25
c27
c39