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Soil archives of chernozems

Půdní archivy v černozemích

Master thesis

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Soil archives of chernozems

Cíle práce ***The aims of work***

Půdy v sobě obsahují mnoho složek, které napomáhají získat představu o vývoji krajiny a vegetačního krytu v minulosti. Pro výzkum paleoenvironmentálních podmínek vývoje černozemí již bylo použito mnoho metodických přístupů. Cílem této práce je provést srovnání výsledků analýzy půdní organické hmoty různými metodami na vzorcích půd ze střední Evropy a USA.

The soil contains many components that help to get an idea of the formation of the landscape and the vegetation cover in the past. Several methodological approaches have already been used to research the paleoenvironmental conditions of the formation of chernozems. The aim of the thesis is to compare the results of qualitative analysis of soil organic matter by different methods on soil samples from the USA and Central Europe.

Použité pracovní metody, zájmové území, datové zdroje

Methods, study area, data sources

Minnesota, USA; střední Evropa; příprava vzorků, analýza NIRS, statistické zpracování hodnot NIRS a stabilních izotopů

Minnesota, USA; Central Europe; sample preparation, NIRS analysis, statistical analysis of NIRS and stable isotope values

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I declare that this thesis is my own work and all used sources of information and literature are properly acknowledged. Neither this thesis nor a substantial part of it was ever used to earn a different or the same academic degree.

In Prague, 15. 8. 2018

Signature:

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Special thanks go to my family for immense emotional, mental and material support.

Abstract: Soil archives provide valuable information on the past of the environment, in particular vegetation history (Costantini 2018). The aim of this thesis is study the feasibility of using the modern method of near-infrared spectroscopy to study the vegetation history of chernozems, using previously collected databases (Vysloužilová et al. 2015) to extract information from new sites along the grassland-woodland boundary in Minnesota, USA. The results are then analyzed and compared to known information and the results of more traditional approaches.

Key words: soil archives, NIRS, isotopes, chernozems

Abstrakt: Půdní archivy poskytují cenné informace o paleoenvironmentu, obzvláště o vegetační historii (Costantini 2018). Cílem této práce je ověřit použitelnost moderní metody blízké infračervené spektroskopie ke studiu vegetační historie černozemí za účelem získaní informací z nových lokalit podél hranice stepi a lesa v Minnesotě, USA s použitím dříve sestavených databází spektrálních dat (Vysloužilová et al. 2015). Výsledky jsou analyzovány a srovnány se známými skutečnostmi a výsledky tradičnějších metod.

Klíčová slova: půdní archivy, NIRS, izotopy, černozemě

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1. Introduction

Soil archives are an important source of information on the past environment. With the advance of new methods over the past 20 years, there has been a surge of studies focusing on the various components of soil that continues to this day. Soil organic matter is particularly important in this regard and is extensively studied with a wide variety of methods (Schwartz et al. 2015, Costantini et al. 2018)

Chernozems have traditionally been considered to be typical soils of the temperate and hemiboreal continental region, forming on loess under grassland vegetation. This traditional view has been challanged as Chernozems and Chernozem-like soils are currently also found in environments that do not correspond the traditional idea. This is especially true in Central Europe where Chernozems can even be found under woodland land cover. Numerous theories have been proposed (from grazing by large herbivores to human induced fires) that could explain the preservation of Chernozems and Chernozem-like soils in this area, some have been largely debunked while others are still considered plausible (Lorz and Saile 2011, Vysloužilová et al. 2014). Similarly, numerous factors were present along the grassland-woodland boundary in North America that had the potential to influence the development of soils. Wildfires and herbivores are cited most often (Feggestad et al. 2004).

The use of traditional methods such as stable isotopes and novel approaches like near-infrared spectroscopy to study the soil organic matter of Chernozems and Chernozem-like soils in these regions can shed more light on the question of vegetation history of these soils and in turn help us understand the environmental conditions that were present in these regions throughout the Holocene (Ertlen et al. 2015).

2. Soil archives

Environmental archives, regionally also referred to as geoarchives, are natural structures that can be used for the reconstruction of the past environment. Typical examples of environmental archives that have been studied for decades (and more) include sedimentary sequences, palynological sequences, and various paleozoological and paleobotanical records (Grunewald and Scheitauer 2008). In recent years, however, surface soils and in particular Chernozems have been suggested as a potentially highly valuable environmental archive (Terhorst et al. 2015). Integrative studies of the surface and near-surface environment are recognized as an important area of focus by the scientific communities, including geography, geology, biology, pedology and hydrology (Driese and Nordt 2013).

Soil archives are the components of soils, the presence or properties of which can be used to extract information on the history of vegetation, land use and the environment as a whole (Schwartz et al. 2015). They have been referred to as the "memory of the landscape" (Vysloužilová 2015). The attributes of soil, coupled with climate variables, have traditionally been used in agriculture to predict the potential and limitations of land areas to produce food (National Research Council 2001). Paleopedology is a discipline that focuses on the study of soil archives. With the advance of modern research methods, the number of studies focusing on environmental history using soils as the archive has increased (Schwartz et al. 2015, Costantini 2018).

The main advantage of using soils as environmental archives lies in their local nature – i. e. the components are of local origin and the presence of certain soils reflects the local conditions. The organic matter generally originates from plants that were present at the given locality or not far away. Given the fact that soils cover most of the terrestrial surface, it allows for the local paleoenvironment to be studied globally (Schwartz et al. 2015).

The paleoenvironment can also be studied thanks to the knowledge of conditions required for particular pedogenic processes. The mere presence of a certain (paleo)sol can be an important source of information. Some soils only tend to develop under very specific conditions (e. g. Solonetz, Solonchak or even Chernozem) (Schwartz et al. 2015, Costantini 2018).

Dating has long been an issue for researchers in the field of paleopedology. Given the dynamic nature of soils, obtaining reliable radiocarbon dates that can be correctly interpreted, is problematic (Schwartz et al. 2015, Costantini 2018).

2.1. Buried soils

Buried soils are soils that formed in place and were later covered by new material and preserved (Galbraith 2011). Buried soils have long been studied as environmntal archives in conjunction with loess layers in loess-paleosol sequences (Frechen et al. 2003). These have been referred to as some of the most important terrestrial archives (Muhs et al. 2014).

Paleopedology and pedostratigraphy are devoted to the use of buried (and relict) soils in interpreting the record of environmental conditions that are different from those currently observed (Constatntini 2018). Buried soils (and paleosols in general) store information about the environmental conditions present at the time of their development and thus reflect the history of the landscape. This particular nature of paleosols, referred to as “soil memory” (Targulian and Goryachkin 2004, Costantini et al. 2007), makes them particularly valuable (Constatntini 2018).

2.2. Components of soil

A number of components of soil have been used as soil archives. Studying the micromorphological characteristics of soils has been used for decades and has the ability to uncover processes that affected the studied soil, including anthropogenic factors, such as evidence of past tillage. However, the treatment of samples is a time-consuming process and correct interpretation requires experience (Schwartz et al. 2015).

Various chemical analyses can also be used to interpret the environmental history. The availability of phosphorus and other nutrients have an effect on the composition of vegetation in a given location (Chmelíková and Hejcman 2014).

Pedoanthracology studies charcoals preserved in soil. Pieces of charcoal retain the characteristic structure of plant tissue that is used to determine the species the charcoal originated from. Generally, charcoals decay slowly and can be preserved in soil for thousands of years. Given the abundance of carbon in charcoal, carbon dating is often performed. Dating in conjunction with the knowledge of the plant species provides invaluable information on the paleoenvironment (Vysloužilová 2015).

2.3. Soil organic matter

Various methods have been used to study vegetation history and the paleoenvironment as a whole using soil organic matter as the main source of information as it contains characteristic residues of the vegetation supported over several thousand years (Ertlen et al. 2010, Guillet 1979 in Ertlen et al. 2010).

A popular method of discrimination between different forms of land cover is $\delta^{13}C$, that is the ratio of ^{13}C to ^{12}C. This can be matched to the isotopic ratios of various plants depending on their photosynthetic cycles (C_3 vs. C_4). However, this method can only be applied in regions where significant numbers of C4 plants can be found (Schwartz et al. 1984).

Other methods have been used less frequently. These include changes in distribution of monosaccharides (Trouvé et al. 1996 in Ertlen et al. 2010), the composition of lignin and carbohydrates (Guggenberger et al. 1994 in Ertlen et al. 2010) or ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Nierop et al. 2001 in Ertlen et al. 2010). However, the use of these methods is a time-consuming and high-cost approach (Ertlen et al. 2010).

A method popular in a number of fields, including soil science, archeology and paleontology, is the analysis of phytoliths, microscopic forms of biogenic silica in plant tissue (Piperno 2006) that can be found preserved in the fossil record and soils (Carter 1999). Based on the shape of these phytoliths, specific species or groups of plants can be identified, indicating their past presence at the given sites (Bobrova and Bobrov 1998). It has been shown that light intensity can influence cell morphology, the size of cells that reconstructed from phytoliths has been used as an indicator of canopy cover, making it possible to estimate past woodland or grassland cover (Dunn et al. 2015). For example Bobrova and Bobrov

(1998) used phytoliths to reconstruct past vegetation on various surface and buried soils throughout Russia, including Chernozems.

In recent years, analyses of leaf wax derived long chain n-alkanes preserved in soil have proven the capacity to reconstruct past vegetation. Despite this fact, their dynamics and factors affecting their distribution in soils is not fully understood yet and research is still ongoing (Schaefer et al. 2016).

Another modern approach that is used to determine the origin of the soil organic matter is the analysis of molecular biomarkers. Low molecular weight biomarkers can be used to trace both the origin and the stage of the soil organic matter. The method is continuously developed and improved but requires advanced chemical procedures to be performed (Otto and Simpson 2007).

2.3.1. Near-infrared spectroscopy (NIRS)

Near-infrared spectroscopy (NIRS) is a relatively rapid, low-cost, reproducible and non-destructive method. While NIRS has been used for various industrial and research applications for decades, its use for the reconstruction of past vegetation so far has been limited, though numerous authors have used it to characterize the composition of living plants as well as plant litter. Near-infrared spectroscopy was first applied to soils by Bowers and Hanks (1965), paving the way for future research, mostly focused on the estimation of carbon and nitrogen content of soils (Chang et al. 2001, Ertlen et al. 2010).

The mechanism behind using NIRS for the reconstruction of past vegetation is that the NIR absorbance spectra contain a large quantity of information arising from the overtones of vibration, stretching and bending of chemical bonds in the soil organic matter from which they are obtained (Ertlen et al. 2015). This results in a specific recorded NIR spectrum for the composition of each analyzed sample and has been referred to as the concept of fingerprinting (Palmborg and Nordgren 1996).

Near-infrared spectroscopy allows soil organic matter to be studied without time-consuming and often costly processes of chemical extraction, even in soils with low organic content (Barthes et al. 2008). The relative ease and low cost of NIRS make it a useful tool of analyses of large datasets, allowing for large spatial resolution (Viscarra Rossel et al. 2011).

2.3.2. Stable isotopes

Stable isotopes of two elements – carbon and oxygen – extracted from soil organic matter are commonly used to retrieve information on the paleoenvironmental conditions on various scales as well as the dynamics of pedogenesis (Tabor and Myers 2015).

2.3.2.1. Oxygen

Isotopes of oxygen can be used reconstruct temperatures and precipitation in the past (Busacca and Sweeney 2005). The proportion of the ^{18}O isotope is controlled by two factors – temperature and spatial position. A growth in the amount of ^{18}O recorded in precipitation or water vapor is observed with increasing temperature. Similarly, the proportion of ^{18}O increases with a decreasing distance from the ocean which serves as the source of 18O. This effect is observed because ^{18}O tends to condensate more easily compared to ^{16}O, thus it deposited by precipitation closer to the ocean (Fig. 1). A difference in oxygen isotope ratios can also be attributed to changes in precipitation – generally, a higher proportion of ^{16}O may denote increased precipitation (Hasinger et al. 2015).

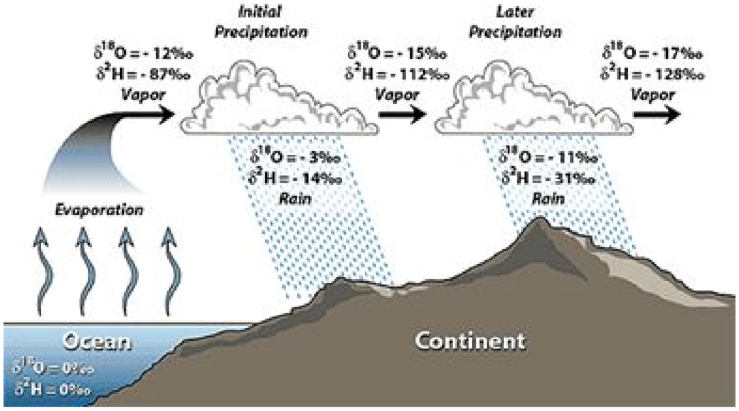


Figure 1: Rainout effect on ^{18}O values (University of Arizona 2018).

2.3.2.2. Carbon

The values of carbon isotopes found in soil organic matter are an important source of information related to the paleoenvironment as well as vegetation history. Using carbon isotope ratios, the relative proportion of plants with different metabolic pathways can be reconstructed (Kaakinen et al. 2006). There are two major types of metabolic pathways – C3 and C4 (Fig. 2). These two types differ in the discrimination of atmospheric $^{13}CO_2$ (Šantrůček, Šantrůčková et al. 2014).

In plants with the C3 metabolic pathway, $\delta^{13}C$ values generally fall in the range of -32‰ to -22‰ of Vienna Pee Dee Belemnite (VPDB), with a mean value of circa -27‰ VPDB, while these values range from approximately -17‰ to -9‰ of VPDB, with mean values of around -13‰ VPDB (Boutton et al. 1998). A third type of metabolic pathway is called CAM, short for Crassulacean Acid Metabolism. This type could be described as a combination of the two major types and the mean value falls between the C3 and C4 types. However, this type of metabolism can only be found in a very small percentage of plants, a typical representative is the genus *Crassula* (Šantrůček, Šantrůčková et al. 2014).

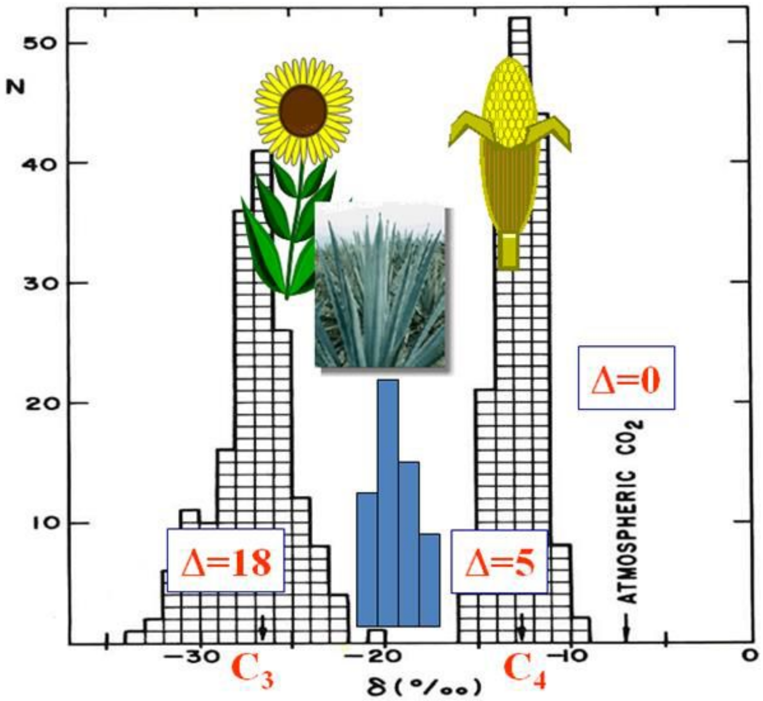


Figure 2 $^{13}CO_2$ discrimination among species in plant groups (Šantrůček et al. 2014).

It is known that climate has a great effect on the performance of these types of metabolism (Schulze et al. 1996 in Pyankov et al. 2010). Thus, each type displays a specific pattern of spatial distribution. C4 plants are able to perform well when their stomata need to be partially closed to prevent loss of water. The distribution of C4 species is correlated with the climate, especially the factors of temperature and precipitation (Pyankov et al. 2010). Many C4 species, mostly grasses, are believed to originate or have large parts of their geographical range in tropical and subtropical regions (Teeri and Stowe 1976). Globally, approximately 3% of terrestrial plant species use the C4 metabolic pathway (Kellogg 2013).

2.3.2.2.1. Europe

Pyankov et al. (2010) summarized the distribution of C4 plants in Europe. The results show a very low percentage of C4 species (predominatly grasses) among all vascular species throughout Europe (Fig. 3). In addition, the percentage of native C4 species among all C4 found in each region was studied. In Central Europe, it is shown that up to 38% of current C4 species are non-native. Given these facts, it is virtually impossible to relevantly estimate the prevalence of grassland in the Holocene using $\delta^{13}C$ values retrieved from soil organic matter throughout Europe.

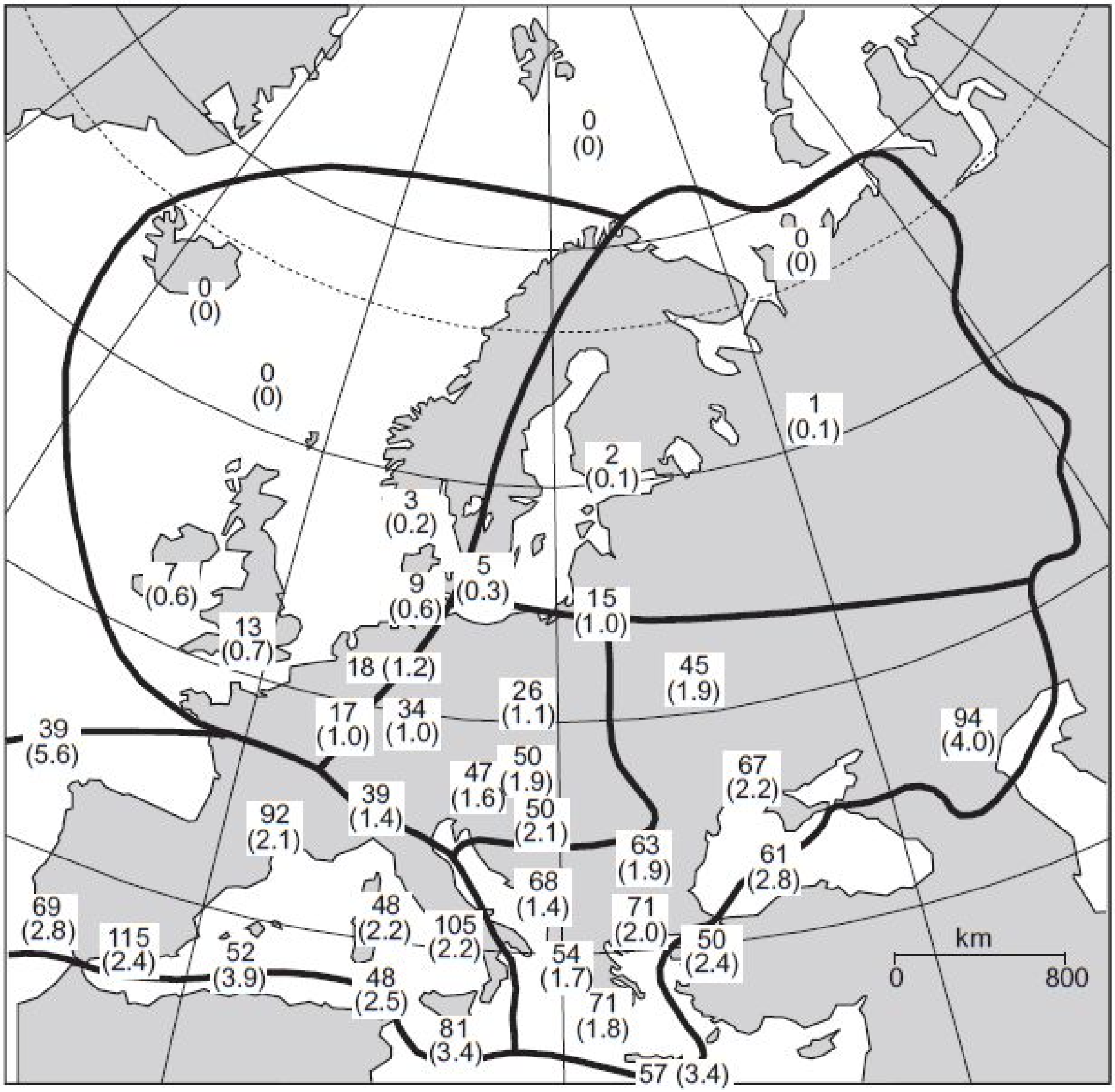


Figure 3 Geographical distribution in Europe of total C4 species; numbers with % C4 species of total vascular species of the respective area in brackets (Pyankov et al. 2010).

2.3.2.2.2. North America

Teeri and Stowe (1976) studied the distribution of C4 grasses (*Poaceae*) of North America. Their results suggest that high minimum temperatures during the growing season have a strong correlation with the relative abundance of C4 grass species in the flora of the 32 studied regions within North America. In the state of Minnesota, the percentage of C4 species among all grasses range from 22% in the north east (Lakela 1965 in Teeri and Stowe 1976) up to 34 to 37% in other parts of the state and the region immediately surrounding it (Fig. 4); Fassett 1951 in Teeri and Stowe 1976, Hartley 1966 in Teeri and Stowe 1976). Using the findings of Teeri and Stowe (1976), i.e. the relatively high proportion of C4 species among grasses in the region, and considering the land cover in the region, it is possible to estimate the prevalence of grassland vegetation in this using δ^{13}C values (Feggestad et al. 2004).

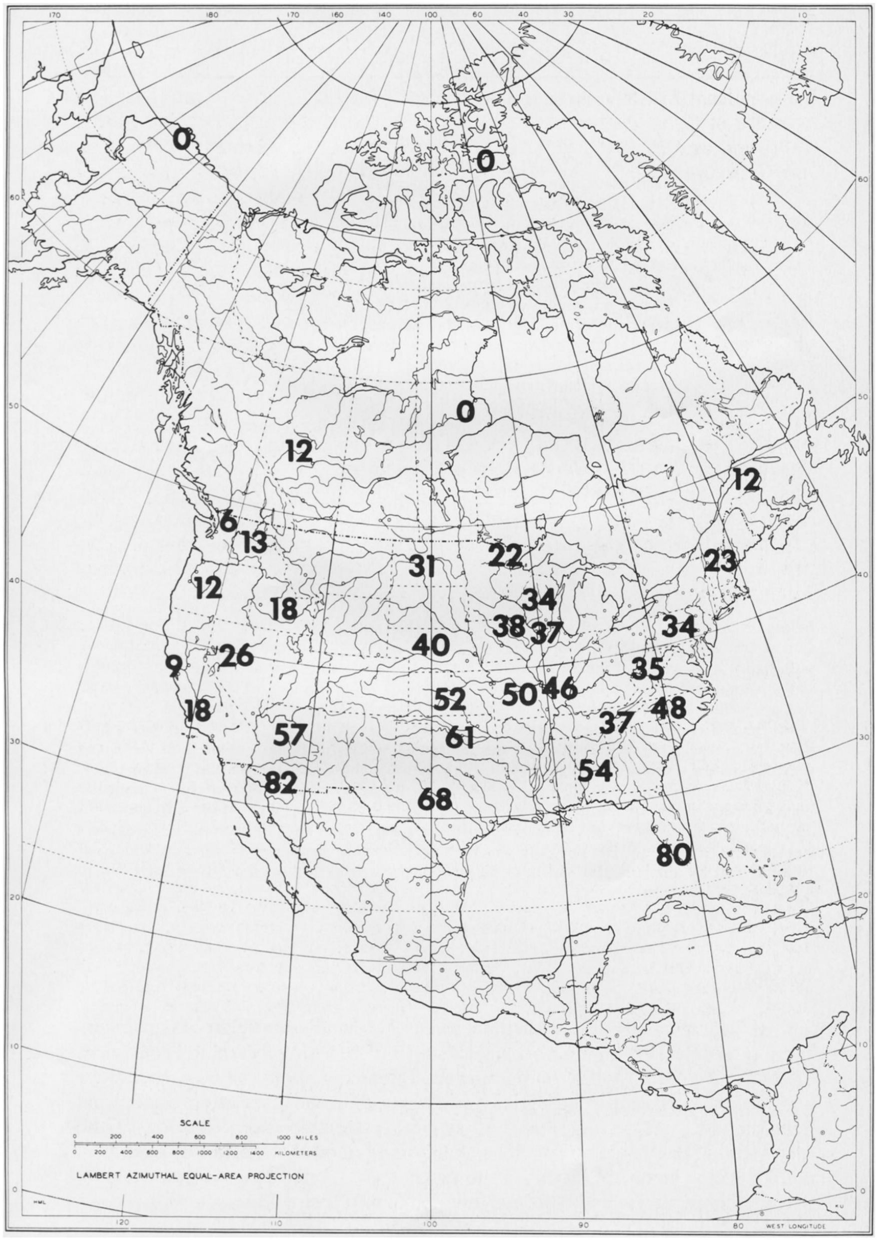


Figure 4: The percent of C4 species in the grass floras of 32 regions in North America (Teeri and Stowe 1976).

3. Chernozem

3.1. Definitons

The World Reference Base for Soil Resources (IUSS Working Group WRB 2015), a taxonomic classification system, defines Chernozems in detail as soils having a chernic horizon (i.e. a relatively thick, well-structured blackish surface horizon, with a high base saturation, high biological activity and with moderate to high content of organic carbon, see below), this horizon is considered diagnostic. In addition, a calcic horizon or a layer with protocalcic properties starting 50 cm or less below the lower limit of the mollic (i.e. a structured surface horizon, dark in color, with a high base saturation and moderate organic matter content; the chernic horizon thus meets the criteria of such horizon) horizon (and if present, above a cemented or indurated layer) as well as a base saturation of fifty percent or more from the soil surface to the calcic horizon or the layer with protocalcic properties, throughout, must be present (IUSS Working Group WRB 2015).

The chernic horizon is described as a thick, well-structured and dark colored surface horizon, with high base saturation, high biological activity and moderate to high organic matter content. It has to meet the following criteria: ≥ 20% (by volume, weighted average) of fine earth; and granular or fine subangular blocky soil structure; and ≥ 1% soil organic carbon; and at least one of the following: in slightly crushed samples a Munsell colour value of ≤ 3 moist, and ≤ 5 dry, and a chroma of ≤ 2 moist, or ≥ 40% (by mass) calcium carbonate equivalent in the fine earth fraction and/or a texture class of loamy sand or coarser, and in slightly crushed samples a Munsell colour value of ≤ 5 and a chroma of ≤ 2, both moist, and ≥ 2.5% soil organic carbon; and ≥ 1% (absolute) more soil organic carbon than the parent material, if parent material is present, that has a Munsell colour value of ≤ 4, moist; and a base saturation of ≥ 50% on a weighted average, throughout the entire thickness of the horizon; and a thickness of at least 25 cm (IUSS Working Group WRB 2015).

A calcic horizon is defined as a horizon with secondary calcium carbonate accumulation in a diffuse form or as discontinuous concentrations (veins, coatings, nodules). Primary carbonates may be present as well. To be diagnosed as calcic, the horizon needs to have a calcium carbonate equivalent in the fine earth fraction of ≥ 15%; and either ≥ 5% (by volume) secondary carbonates or a calcium carbonate equivalent in the fine earth fraction of ≥ 5% higher (absolute, by mass) than that of an underlying layer and no lithic discontinuity between the two layers or both; and does not form part of a petrocalcic horizon; and is at least 15 cm thick (IUSS Working Group WRB 2015).

A layer with protocalcic properties is a layer with permanent secondary carbonates, i. e. not belonging to the soil parent material or other sources such as dust. The carbonate accumulations need to meet the following criteria for a layer to have protocalcic properties: disrupt the soil structure or fabric; or occupy ≥ 5% of the soil volume with masses, nodules, concretions or spheroidal aggregates (white eyes) that are soft and powdery when dry; or cover with soft coatings ≥ 50% of structural faces, pore surfaces or undersides of rock or cemented fragments, thick enough to be visible when moist; or form permanent filaments (pseudomycelia) (IUSS Working Group WRB 2015).

Chernozems are further described as blackish soils rich in organic matter (Fig: 6). The parent material is mostly made up of eolian and reworked eolian sediments (principally loess). Generally, Chernozems can be found in regions with a continental climate with cold winters and hot summers, which are dry at least in late summer; in flat to undulating plains with tall-grass vegetation (hardwood forest especially in the northern transitional zone). Chernozems cover an estimated 230 million ha worldwide, mainly in the mid-latitude steppes of Eurasia and North America (Fig. 5) (IUSS Working Group WRB 2015).

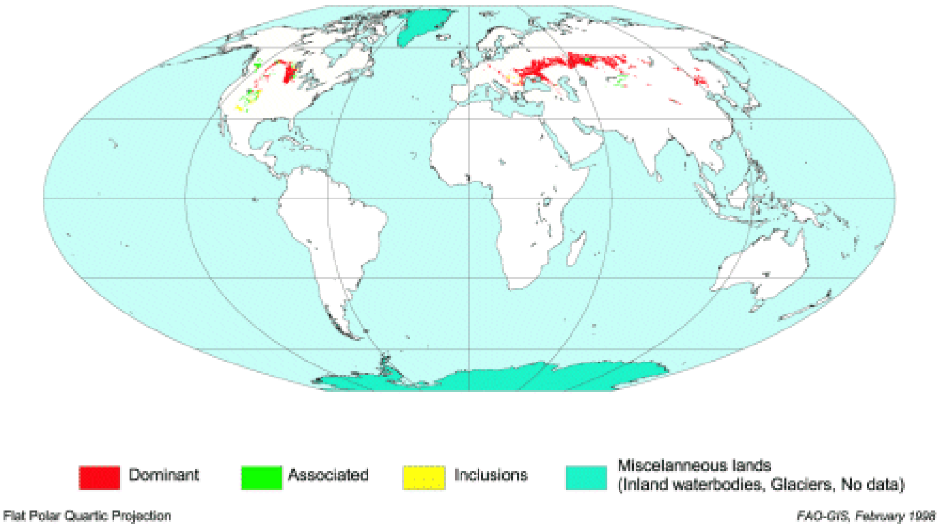


Figure 5: Global distribution of Chernozems (FAO 1998).

Deep Chernozems are commonly ranked among the best soils in the world for agricultural use. Wind and water erosion are best prevented by preserving the favorable soil structure through timely cultivation and careful irrigation at low watering rates. Fertilizers are frequently used for high yields. The principal crops grown on Chernozems are wheat, barley and maize, alongside other food crops and vegetables. Part of the Chernozem area is used for livestock grazing. In the northern temperate belt, the possible growing period is limited and the principal crops grown are wheat and barley, in places in rotation with vegetables. Maize and sunflower are widely grown in the warm temperate belt. Maize production tends to stagnate in drier years unless adequate irrigation is provided (IUSS Working Group WRB 2015).

The first recorded use of the term Chernozem (from Russian *черная/chernaya,* black; and *земля/zemlya*, ground or land) was in 1645 by Salmon Gubert (Reintam 2001). Other early uses of the term are attributed to Lomonosov in 1765 (Krupenikov et al. 2011) or Akonin in 1771 (Kubiena 1953 in Vysloužilová et al. 2016). The term was used by Dokuchaev (e.g. 1883) to describe the typical soils of the steppe in continental Russia (IUSS Working Group WRB 2015). Dokuchaev's extensive research on these soils led to the creation of a new science, now known as pedology or soil science. The original aim of his research was to determine why some soils are more fertile than others for the purpose of taxation (Johnson and Schaetzl 2015).

Figure 6: Chernozem near Karcag, Hungary (photo: author).

3.2. Correlation of Terminology

Assigning soils commonly called Chernozems in a number of regions (including Central Europe) this name according to the WRB classification can prove difficult (Eckmeier et al. 2007). Local soil classification systems often emphasize pedogenetic factors rather than a simple description of morphology as is the case with the international WRB system. On top of that, the WRB definition is based on typical zonal Chernozems, making identification of these soils as Chernozems, especially at the extremes of its geographical distribution, complicated (Zádorová and Penížek 2011). The term Chernozem-like soils has been used to describe soils that share the pedogenic features (including the presence of a dark chernic horizon, calcareous parent material, high base saturation or strong biological activity) but cannot be classified as Chernozems in the WRB classification (Vysloužilová et al. 2015).

3.2.1. World Reference Base for Soil Resources (WRB)

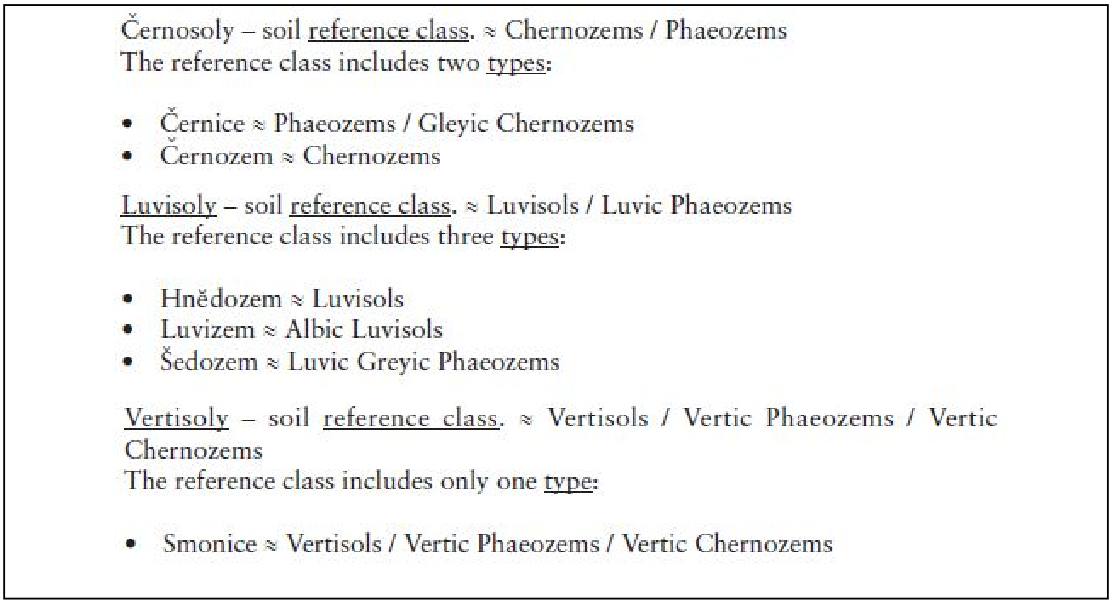
The World Reference Base for Soil Resources has its roots in the original legend of the FAO-UNESCO World Soil Map (1974). The original aim was to evaluate global soil resources and to help correlate national classifications in a basic manner and was largely a compilation of historically recognized soil archetypes without the ambition to replace the more detailed national systems. It has since evolved into a detailed system that can be applied globally at various scales (Krasilnikov et al. 2009).

3.2.2. Czech Republic

Unlike the WRB system, The Soil Taxonomic Classification System of the Czech Republic (Němeček et al. 2011) does not put criteria on the position of the calcic horizon in Chernozem, neither is there a requirement of the concentration of carbonates within 50 cm of the lower limit of the mollic horizon. Still, 91% of soils classified as Chernozems in the Czech system were found to fit the criteria of Chernozems according to the WRB system (Zádorová and Penížek 2011).

The newer versions (post-2000) were made with the correlation to the WRB system in mind. However, at the highest level of hierarchy, WRB defined Chernozems and Chernozem-like soils in general can fall into a number of *reference classes* (Fig. 7) (Krasilnikov et al. 2009).

The WRB defined Reference Soil Group of Chernozems corresponds to various soils in other European classification systems, for example Kalktschernoseme (Germany), Chernosols (France) (IUSS Working Group WRB 2015).



Černosoly – soil reference class. ≈ Chernozems / Phaeozems
The reference class includes two types:

- Černice ≈ Phaeozems / Gleyic Chernozems
- Černozem ≈ Chernozems

Luvisoly – soil reference class. ≈ Luvisols / Luvic Phaeozems
The reference class includes three types:

- Hnědozem ≈ Luvisols
- Luvizem ≈ Albic Luvisols
- Šedozem ≈ Luvic Greyic Phaeozems

Vertisoly – soil reference class. ≈ Vertisols / Vertic Phaeozems / Vertic Chernozems
The reference class includes only one type:

- Smonice ≈ Vertisols / Vertic Phaeozems / Vertic Chernozems

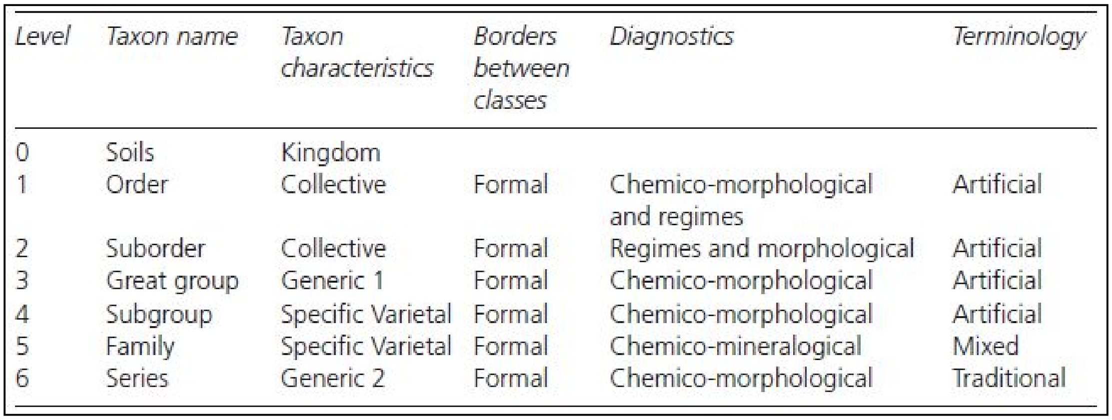
Figure 7: Correlation of WRB and Czech systems for Chernozem-like soils (Krasilnikov et al. 2009).

3.2.3. United States of America

The USDA Soil Taxonomy is considered by some to be the most precisely developed classification currently in use and along with the United States is used officially in dozens of countries. Contrary to many other national classification systems, the object of classification is the profile (or a small representative volume), not the processes and factors of soil formation. The classification is based on quantitative diagnostic soil properties. An unusual feature of the USDA Soil Taxonomy is the requirement to measure or estimate information on temperature and moisture regimes of soils for full classification (Krasilnikov et al. 2009).

There are six levels in the USDA Soil Taxonomy (from highest to lowest): Orders, Suborders, Great Groups, Subgroups, Families and Series (Fig. 8). The Orders divide soils based on properties or conditions resulting from, or reflecting, major soil-forming processes that are relatively stable in time. The Suborders are soils (within an Order) that have certain properties or conditions that are major controls (or reflect such controls) on the current set of soil-forming processes. More dynamic features are selected as evidence of influences on pedogenesis at this level. The Great Groups are soils (within a Suborder) having additional properties that constitute subordinate or additional controls (or reflect such controls) on the current set of soil-forming processes. The Subgroups (within a Great Group) group together soils having properties resulting from a blending or overlapping of sets of processes that cause one kind of soil to develop from, or towards, another kind of soil. These Suborders are essentially links to other classes in higher levels of the taxonomy. Families can be defined as soils (within a Subgroup) having properties that are often indicative of the potential for further pedogenic development, i. e. the chemical and physical capacity to change (Krasilnikov et al. 2009).

Soils generally corresponding to WRB Chernozems were formerly called Calcareous black soils in the USDA Soil Taxonomy and mostly belong now to several Suborders (especially Udolls) of the Mollisols (IUSS Working Group WRB 2015). However, a more detailed look (Fig. 9) reveals a considerably more complex scheme for the correlation of Chernozems and Chernozem-like soils (i. e. Phaeozems) and the USDA Soil Taxonomy (Krasilnikov et al. 2009).



Level	*Taxon name*	*Taxon characteristics*	*Borders between classes*	*Diagnostics*	*Terminology*
0	Soils	Kingdom			
1	Order	Collective	Formal	Chemico-morphological and regimes	Artificial
2	Suborder	Collective	Formal	Regimes and morphological	Artificial
3	Great group	Generic 1	Formal	Chemico-morphological	Artificial
4	Subgroup	Specific Varietal	Formal	Chemico-morphological	Artificial
5	Family	Specific Varietal	Formal	Chemico-mineralogical	Mixed
6	Series	Generic 2	Formal	Chemico-morphological	Traditional

Figure 8: USDA Soil Taxonomy leveles (Krasilnikov et al. 2009)

Mollisols – soil order. ≈ Chernozems / Phaeozems / Kastanozems / Mollic Solonetz
Albolls – soil suborder. ≈ Luvic Phaeozems (Albic, *Ferric*) / Mollic Solonetz (Albic, *Ferric*)
The following great groups are distinguished within the suborder:

- ***Argialbolls*** ≈ Luvic Phaeozems (Albic, *Ferric*)
- ***Natrialbolls*** ≈ Mollic Solonetz (Albic, *Ferric*)

Aquolls – soil suborder. ≈ Gleyic Chernozems / Gleyic Phaeozems / Mollic Gleyic Solonetz
The following great groups are distinguished within the suborder:

- ***Argiaquolls*** ≈ Luvic Gleyic Chernozems / Luvic Gleyic Phaeozems / Luvic Gleyic Kastanozems
- ***Calcaquolls*** ≈ Gleyic Chernozems / Gleyic Kastanozems
- ***Cryaquolls*** ≈ Gleyic Chernozems (*Gelic*) / Gleyic Phaeozems (*Gelic*) / Gleyic Kastanozems (*Gelic*)
- ***Duraquolls*** ≈ Petroduric Gleyic Chernozems / Petroduric Gleyic Phaeozems / Petroduric Gleyic Kastanozems
- ***Endoaquolls*** ≈ Gleyic Phaeozems
- ***Epiaquolls*** ≈ Stagnic Phaeozems
- ***Natraquolls*** ≈ Mollic Gleyic Solonetz

Cryolls – soil suborder. ≈ Chernozems / Phaeozems / Mollic Solonetz
The following great groups are distinguished within the suborder:

- ***Argicryolls*** ≈ Luvic Chernozems (Gelic) / Luvic Phaeozems (Gelic) / Luvic Kastanozems (Gelic)
- ***Calcicryolls*** ≈ Calcic Chernozems (Gelic) / Calcic Kastanozems (Gelic)
- ***Duricryolls*** ≈ Petroduric Chernozems (Gelic) / Petroduric Phaeozems (Gelic) / Petroduric Kastanozems (Gelic)
- ***Haplocryolls*** ≈ Chernozems (Gelic) / Phaeozems (Gelic) / Kastanozems (Gelic)
- ***Natricryolls*** ≈ Mollic Solonetz (*Gelic*)
- ***Palecryolls*** ≈ Luvic Chernozems (*Gelic*) / Luvic Phaeozems (*Gelic*) / Luvic Kastanozems (Gelic)

Rendolls – soil suborder. ≈ Rendzic Leptosols / Rendzic Phaeozems
The following great groups are distinguished within the suborder:
- ***Cryrendolls*** ≈ Rendzic Leptosols (*Gelic*) / Rendzic Phaeozems (*Gelic*)
- ***Haprendolls*** ≈ Rendzic Leptosols / Rendzic Phaeozems

Udolls – soil suborder. ≈ Chernozems / Phaeozems / Kastanozems / Mollic Solonetz
The following great groups are distinguished within the suborder:

- ***Argiudolls*** ≈ Luvic Chernozems / Luvic Phaeozems
- ***Calciudolls*** ≈ Calcic Chernozems / Petrocalcic Chernozems / Petrocalcic Phaeozems
- ***Hapludolls*** ≈ Chernozems / Phaeozems
- ***Natrudolls*** ≈ Mollic Solonetz
- ***Paleudolls*** ≈ Luvic Chernozems (*Profondic*) / Luvic Phaeozems (*Profondic*) / Chernozems (Bathypetrocalcic) / Phaeozems (Bathypetrocalcic)
- ***Vermiudolls*** ≈ Vermic Chernozems / Vermic Phaeozems

Ustolls – soil suborder. ≈ Chernozems / Kastanozems / Phaeozems / Mollic Solonetz
The following great groups are distinguished within the suborder:

- ***Argiustolls*** ≈ Luvic Chernozems / Luvic Kastanozems
- ***Calciustolls*** ≈ Calcic Chernozems / Calcic Kastanozems / Petrocalcic Chernozems / Petrocalcic Kastanozems / Petrocalcic Phaeozems / Gypsic Chernozems / Gypsic Kastanozems
- ***Durustolls*** ≈ Petroduric Chernozems / Petroduric Kastanozems / Petroduric Phaeozems
- ***Haplustolls*** ≈ Chernozems / Kastanozems / Phaeozems
- ***Natrustolls*** ≈ Mollic Solonetz
- ***Paleustolls*** ≈ Luvic Chernozems (*Profondic*) / Luvic Kastanozems (*Profondic*) / Luvic Phaeozems (*Profondic*) / Chernozems (Bathypetrocalcic) / Kastanozems (Bathypetrocalcic) / Phaeozems (Bathypetrocalcic)
- ***Vermiustolls*** ≈ Vermic Chernozems / Vermic Kastanozems / Vermic Phaeozems

Xerolls – soil suborder. ≈ Kastanozems / Chernozems / Mollic Solonetz
The following great groups are distinguished within the suborder:

- ***Argixerolls*** ≈ Luvic Kastanozems / Luvic Chernozems
- ***Calcixerolls*** ≈ Calcic Kastanozems / Calcic Chernozems / Gypsic Kastanozems / Gypsic Chernozems
- ***Durixerolls*** ≈ Duric Kastanozems / Duric Chernozems
- ***Haploxerolls*** ≈ Kastanozems / Chernozems
- ***Natrixerolls*** ≈ Mollic Solonetz
- ***Palexerolls*** ≈ Luvic Kastanozems (*Profondic*) / Luvic Chernozems (*Profondic*) / Petrocalcic Kastanozems / Petrocalcic Chernozems

Figure 9: Correlation of the WRB and USDA systems for Chernozem-like soils (Krasilnikov et al. 2009)

3.3. Evolution

Chernozems and Chernozem-like soils generally develop on loamy calcareous materials, usually loess but they can also be found on other parent materials, including lake sediments or glacial till. Typically, they occur in temperate continental regions and the typical distinguishing mark is the very dark (blackish) A horizon (IUSS Working Group WRB 2015).

Perhaps the most important pedogenetic processes of Chernozems are the long maturation of soil organic matter (SOM) and the formation of weathering complex (Duchaufor 1977 in Vysloužilová et al. 2016). The origin of most of the soil organic matter can be traced to the decomposition process of plants as well as the release of organic material from roots. In part it consists of microbial mass (Guggenberger 2005). The soil organic matter passes into the soil itself through the root system or litter (Vysloužilová et al. 2016).

Most of the soil organic matter is made up of decomposed roots or the organic material released from roots. The above ground parts of plants contribute considerably less to the total amount of the soil organic matter. Grassland species tend to develop deep root systems leading to high organic matter rates in a thick layer. The development of the root system is largely dictated by climate, in this case the continental climate causes the plants to exploit large volumes of soil (Vysloužilová et al. 2016).

The seasonal fluctuations in a continental climate result in a distinctive soil climate. Humification as well as mineralization are controlled by the climate. The most favorable conditions for humification occur in the spring due to a marked increase in soil moisture caused by snow melt allowing the intruding water to create an aerobic environment for a limited period of time. In this environment, water soluble material produced by plant roots (notably grassland species) is accumulated. During the winter and summer this process is significantly impaired (Bridges 1970).

The notion of Chernozems as steppe soils has been questioned, particularly in Central Europe where the climate does not correspond to that commonly associated with Chernozem development, yet large areas covered by these soils can be found in the region (Vysloužilová et al. 2014). While it is widely believed that Chernozems in this area formed under climatic conditions different from those currently observed, it remains unclear how these soils were preserved until the present day (Eckmeier et al. 2007).

An important aspect of the Chernozem development question is undoubtedly its vegetation history. This is particularly highlighted in regions such as Central Europe or the tallgrass prairie areas situated to the east of the Great Plains region of North America. Rainfall in these regions is sufficient for the development of woodland (and has been for much of the Holocene). However, soils such as Chernozems that are commonly associated with a more continental climate and grassland ecosystems have been preserved in both areas with no major changes to their characteristics (Feggestad et al. 2004, Lorz and Saile 2011).

In Central Europe, the existing Chernozems are believed to have been formed in the early Holocene when the climate was considerably more continental. The preservation of steppe conditions was generally thought to have been because of the conservation of grassland patches throughout the Holocene in the driest areas, later assisted by anthropogenic factors, namely agriculture (Lorz and Saile 2011). Recently, other factors have been suggested that include (over)grazing by large herbivores (Lorz and Saile 2011) or human induced fires (Eckmeier et al. 2007).

Along the eastern flanks of the tallgrass prairie of North America, grassland is believed to have been preserved for much of the Holocene by grazing and fire. However, wildfires in this region tend to occur at a far greater frequency than in Central Europe. The black carbon content in American Chernozems is also substantially higher compared to Europe (Skjemstad et al. 2002). Lorz and Saile (2011) do not see fires as an important factor in the formation of European Chernozems. In contrast to Europe, ancient agriculture is not believed to have played a major role in Chernozem conservation. In both regions, grass dominated phytocenoses with a sparse tree cover of mostly various oak (*Quercus*) species were once present on Chernozems but intensified agriculture has led to a large scale destruction of these natural or semi-natural ecosystems (NatureServe 2018).

However, the tree cover density in the past remains controversial (Lorz and Saile 2011).

Vysloužilová et al. (2014) have concluded that Central European Chernozems may persist and possibly even develop under woodland. The stability of the organo-mineral complex is credited with enabling the preservation of Chernozem pattern of development for a considerabconsiderable period of time after the establishment of woodland vegetation.

4. Material and methods

4.1. Geographical setting

4.1.1. Minnesota, USA

The studied sites are located in the northern part of the U.S. state of Minnesota along the grassland-woodland ecotone as defined in 19th century land surveys (fig. 10) (Kasmerchak et al. 2018). Most locations are within the Central geologic region of the state as defined by Sansome (1983) while some span the boundary of the Central and Northwestern regions. The Northwestern region is mostly made up of the lakebed of the glacial Lake Agassiz. In many parts of this region, well-developed Mollisols (USDA) providing favorable conditions for agriculture can be found on lake deposits that are up to 50 meters deep. Till plains and other glacial ladndforms are characteristic of the Central region (Sansome 1983).

The studied soils formed on glacial till or sediment displaying similar loamy and calcareous characteristics of the Des Moines ice sheet lobe and the St. Louis sublobe. The material was deposited during the last glaciation, meaning the soil parent material and its age are believed to be relatively constant at all the studied sites (Kasmerchak et al. 2018). Modern mean annual precipitation ranges from 735 mm for the eastern sites, decreasing to 622 mm towards the west. Mean annual temperature ranges from 3.9 °C to 4.1 °C (tab. 1; NOAA 1981–2010 climate normal; Kasmerchak et al. 2018). The climate is humid continental, type Dfb (also called hemiboreal; Köppen 1936).

Major differences in soil morphology are detected along the roughly north-south oriented grassland to woodland transition in northern Minnesota. It is known that this boundary shifted considerably in the Holocene (Kasmerchak et al. 2018). Past research in the area revealed that grassland first expanded to the east in the early Holocene before retreating westward over the past 4,000 years. The primary force behind this shift is believed to have been climate change (Bradbury et al. 1993 in Kasmerchak et al. 2018). Soils along the transition formed on similar parent material. However, soils that are believed to be of forest morphology (tab. 2), mostly equivalent to Luvisols under the WRB classification containing clay-enriched Bt horizons, can be clearly differentiated from soils with transitional or grassland morphologies. Soils with grassland morphology (tab. 2) display relatively uniform clay content throughout the profile (Kasmerchak et al. 2018). Of particular interest for this thesis are the soils classified as Mollisols (tab. 2), equivalent to Chernozems and types with related morphology under the WRB classification. These soils were identified by Kasmerchak et al. (2018) as soils with transitional or grassland morphologies.

site	MAT (°C)	MAP (mm)
E-133	4.8	735
E-27	4.8	735
E-73	4.8	735
E-215	4.1	690
W-151	4.1	690
W-44	4.1	690
W-238	3.9	708
W-171	3.9	708
W-39	3.9	708
W-70	3.9	622
W-22	3.9	622
P-26	3.9	622
P-50	3.9	622
P-79	3.9	622
P-67	3.9	622
P-98	3.9	622

Table 1: Climatic data for studied sites (data from Kasmerchak et al. 2018)

site	longitude	latitude	morphology	USDA order	WRB type	current vegetation	history of cultivation	vegetation history
E-133	-93.8497	47.03431	forest	Alfisol	Luvisol	forest	no	forest
E-27	-93.7688	47.62886	forest	Alfisol	Luvisol	forest	no	forest
E-73	-93.8411	47.43515	forest	Alfisol	Luvisol	forest	no	forest
E-215	-94.0864	47.7435	forest	Alfisol	Luvisol	forest	no	forest after ca. 4 ka
W-151	-94.7187	47.74939	forest	Alfisol	Luvisol	forest	no	forest after ca. 4 ka
W-44	-94.7336	47.64156	forest	Alfisol	Luvisol	forest	no	forest after ca. 4 ka
W-238	-95.2853	47.71518	transitional	Alfisol	Luvisol	forest	no	forest-grass. trans.
W-171	-95.3836	47.63112	transitional	Alfisol	Luvisol	forest	no	forest-grass. trans.
W-39	-95.4885	47.56403	transitional	Alfisol	Luvisol	forest	no	forest-grass. trans.
W-70	-96.132	47.59789	transitional	Alfisol	Luvisol	forest	no	forest-grass. trans.
W-22	-96.1537	47.49365	transitional	Mollisol	Chernozem/Phaeozem	forest	no	forest-grass. trans.
P-26	-95.7954	47.36856	grassland	Mollisol	Chernozem/Phaeozem	grasses (non-native)	possibly (prior to 1950)	grassland
P-50	-95.8511	47.25305	grassland	Mollisol	Chernozem/Phaeozem	grasses (non-native)	possibly (prior to 1950)	grassland
P-79	-95.8722	47.57048	grassland	Mollisol	Chernozem/Phaeozem	grasses (non-native)	yes (prior to 1993)	grassland
P-67	-96.0934	47.24124	grassland	Mollisol	Chernozem/Phaeozem	grasses (non-native)	yes (prior to 2000)	grassland
P-98	-96.1441	47.16555	grassland	Mollisol	Chernozem/Phaeozem	forest	no	grassland

Table 2: Description of studied sites by Kasmerchak et al. (2018).

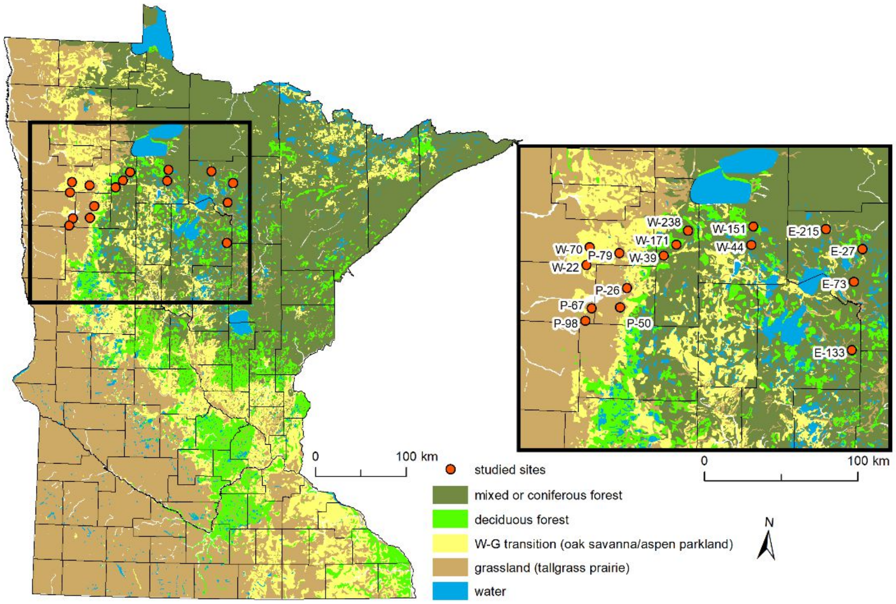


Figure 10:Studied sites in Minnesota, USA.

4.2. Reference library (Europe)

The reference database is a modified version of the database collected by Vysloužilová et al. (2015) throughout the Chernozem belt of Europe, mostly focused on Central Europe. The aim was to build the database using primarily sites with natural or semi-natural vegetation as they may better reflect the natural development of soils. The number of sites fulfilling this criteria is rather limited in this densely populated region where most Chernozems and Chernozem-like soils are exploited as arable land. Most of the soils come from the Czech Republic, Slovakia and Hungary. Some sites are located in France, Ukraine and Russia. There are 23 sites in total (Fig. 11, Tab. 3) (Vysloužilová et al. 2015, Strouhalová et al. 2018).

All but two of the sites (BRC, POP) are currently non-cultivated. A number of sites are under forest vegetation (Tab. 3). The database is mostly made up of soils classified as chernozems in the respective national classification systems but do not necessarily meet the criteria for Chernozem using the WRB system, the soils do however generally share the pedogenic features of Chernozems, including the presence of a dark chernic horizon, calcareous parent material, high base saturation or strong biological activity. The term Chernozem-like soils is thus better suited to avoid confusion with soils meeting the strict WRB definition (Vysloužilová et al. 2015, Strouhalová et al. 2018). The climate ranges from temperate oceanic (Cfb) for the western sites, transitioning to humid continental (Dfb; also known as hemiboreal) in the east (Köppen climate classification, Köppen 1936).

The sites were also chosen with respect to the stability of land cover. Information on land use in the past from the Second Military Survey of the Austrian Empire, dating to 1836–1840 in Moravia (eastern Czech Republic), 1842–1852 in Bohemia (western Czech Republic) and 1819–1869 in Slovakia and Hungary. The maps are available online (mapy.cz, archivportal.arcanum.hu). For the territory of France, the Cadaster Survey and the Archives of National Forest Office (1860 to present) were used (Ertlen and Schwartz 2010 in Vysloužilová 2015). Literature review was used to determine the vegetation history of sites in Russia and Ukraine (Jelenska et al. 2008 in Vysloužilová et al. 2015, Khitrov et al. 2013 in Vysloužilová et al. 2015; Vysloužilová et al. 2015).

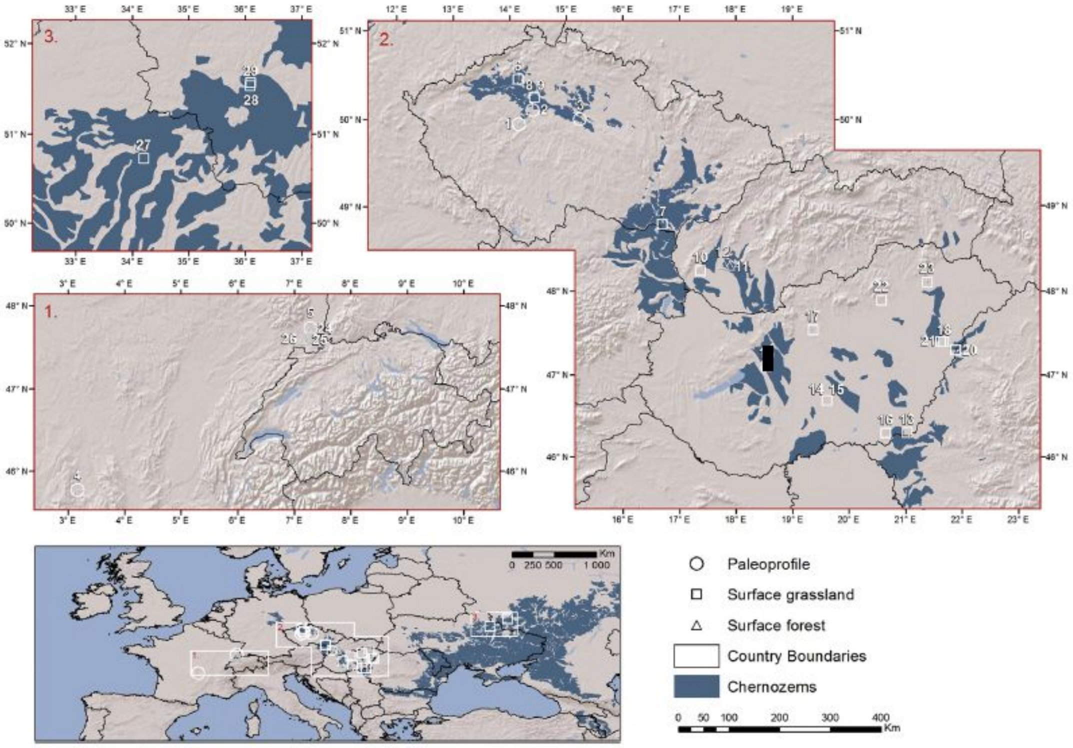


Figure 11: Sites used for the construction of reference library (6-BRO, 7-BUL, 8-KOC, 9-KOR, 10-SEN, 11-BAB, 12-SEN, 13-HUB, 14-HUU, 15-HUUD, 16-HUC, 17-HUM, 18-HUS, 20-HUP, 21-HUH, 22-HUR, 23-HUT, 24-CAR, 25-HIR, 26-DID, 27-MIK, 28-KUR, 29-STR) (Vysloužilová et al. 2015 – modified)

site	country	code	vegetation	soil	parent material
Babský les	Slovakia	BAB	woodland	Chernozem	loess
Battonya-Gulya Gyep	Hungary	HUB	grassland	Chernozem	loess
Brozany	Czech Rep.	BRO	grassland	Chernozem	loess
Bugac puszta	Hungary	HUUD	grassland	Arenic Chernozem	sandy loess
Bugac puszta -dune	Hungary	HUU	grassland	Arenic Chernozem	sandy loess
Bulhary	Czech Rep.	BUL	woodland	Chernozem	loess
Csikóspuszta	Hungary	HUC	grassland	Chernozem	loess
Gallenhölzchen	France	DID	woodland	Calcic Cambisol	loess
Dubník	Slovakia	DUB	woodland	Chernozem	loess
Hajdubagos	Hungary	HUH	grassland	Chernozem	sandy loess
Breiholtz	France	HIR	woodland	Calcic Cambisol	loess
Kopeč - rendzine	Czech Rep.	KOR	grassland	Calcic Leptosol	loess on basalt
Kopeč - chernozem	Czech Rep.	KOC	grassland	Chernozem	loess
Kursk	Russia	KUR	grassland	Chernozem	loess
Mezőföld - B. - valley	Hungary	HUM	grassland	Chernozem	loess
Mikhailovska tselina	Ukraine	MIK	grassland	Chernozem	loess
Pocsaj	Hungary	HUP	grassland	Chernozem	loess
Sáránd	Hungary	HUS	grassland	Arenic Chernozem	sandy loess
Senec	Slovakia	SEN	woodland	Chernozem	loess
Stoeffelhag	France	CAR	woodland	Calcic Cambisol	loess
Streletsk	Russia	STR	grassland	Chernozem	loess
Tard	Hungary	HUR	grassland	Luvic Chernozem	loess
Tokaj	Hungary	HUT	grassland	Calcic Cambisol	loess

Table 3: Sites used in the reference database (data from Vysloužilová et al. 2015)

4.3. Sampling

4.3.1. Minnesota, USA

The samples were provided by Mason, Kasmerchak et al. (2018). The sites were distributed evenly across four zones as defined by Kasmerchak et al. (2018): prairie, believed to be covered by grassland vegetation throughout the Holocene; transition zone; grassland replaced by forest after 4 ka; forests, with woodland cover throughout the Holocene.

The samples were collected in areas defined by targeted soil mapping units on slopes of less than 3.5°. The targeted mapping units were the predominant well-drained soil in each unit. Sites in the forest, forest after 4 ka and transitional zones were all under forest at the time of sampling and are believed not to have been cultivated. Sites selected for this thesis from these zones are only represented by a single sample from the top horizon of each site, with the exception of site W-22 in the transitional zone. The reason is that (with the exception of site W-22) these soils are classified as Luvisols, thus of limited interest for this study (tab. 2). Of the prairie (grassland) zone, 4 sites were under perennial non-native grass cover at the time of sampling and all of them share a possible history of cultivation of up to several decades. However, none were cultivated within the last 20 years before sampling, based on a combination of landowner information and aerial photography. A single site in the grassland soil was under aspen (Populus tremuloides) forest that likely replaced grassland within the past few hundred years (Kasmerchak et al. 2018). A total of 35 samples from 16 sites (tab. 5) were used for this thesis.

The sampling sites were selected from wider sets of randomly selected points in the targeted mapping units (see above) based on a number of factors – ease of access, landowner permission and lack of recent disturbances. The upper soil profile was sampled at 25 random points at each site. The sampling pit was then placed at the point where morphological properties (e.g. A horizon thickness) were the closest to the median of the site. Each pit was then uncovered into the C horizon, described and sampled by subhorizon. Four of the grassland (prairie) zone sites were sampled with three replicate hydraulic soil probe cores (7.6 cm in diameter) each instead of a pit. Material from the full thickness of horizons and two or three walls of the pit is combined in each sample (Kasmerchak et al. 2018).

4.3.2. Reference library

The samples were collected at 24 sites in the temperate region of Europe spanning from eastern France to western Russia by Vysloužilová et al. (2014) (tab. 3, fig. 11). The sampling protocol devised by Ertlen et al. (2010) was used. The sites were selected based on the presence of vegetation documented as stable over the past 150 years. At each reference site 15 to 30 samples of approximately 50 g were collected from the topsoil (0 to 4 cm) in a steel cylinder at 2 m intervals on three parallel lines spaced 4 m apart. Given the stable vegetation at all sites, the soil organic matter in the topsoil is believed to come nearly exclusively from the observed vegetation. A total of 427 samples were used. The reference samples were dried at 40 °C in an oven over 7 days and sieved to pass through a 2 mm mesh before NIRS spectra were collected (Ertlen et al. 2010, Vysloužilová et al. 2014).

4.4. Near-infrared spectroscopy

4.4.1. Acquisition and pretreatment of spectra

35 sieved (to 2 mm) soil samples (tab. 5) of approximately 20 g each from 16 sites in northern Minnesota (tab. 2) provided by Mason, Kasmerchak et al. (2018) were oven dried at 40 °C. Each sample was then placed in a rotating cup with a quartz window (90 mm in diameter) and scanned from 10,000 to 4,000 cm^{-1} with an 8 cm^{-1} resolution (in order to obtain a data matrix of a manageable size) using an Ft-NIR Frontier spectrometer (PerkinElmer, USA) with a CaF_2 beamsplitter, an integrated sphere an an Ingass detector. Each spectrum was measured out of the average of 90 scans performed in a single session from different parts of the sample, made possible by the rotating cup technology. The measurements were performed at a laboratory of the University of Strasbourg, France. Each spectrum was measured as the common logarithm of the inverse of reflectance (R), that is absorbance (A) illustrated by the following formula (Ertlen et al. 2010):

$$A = log10(1/R)$$

The obtained spectra were then analyzed with a reference library of the spectra of 427 samples from 24 sites throughout Europe created by Vysloužilová et al. (2015) using the methodology of Ertlen et al. (2010).

The data matrix with 751 columns was standardized using the Standard Normal Variate (SNV) function using The Unscrambler X software (version 10.3) with the aim of subtracting the average absorbance of the spectra from all the spectra, thus reducing the influence of the quantity of soil organic matter and the of the lack of homogeneity of particle size distribution. It has been shown that this transformation has the potential to highlight qualitative information found in the spectra. First order derivative transformation with a gap of 9 was then applied with the goal of being able to extract the sought after information, that is the origin of the soil organic matter – grassland or woodland (Shenk et al. 2001 in Vysloužilová et al. 2015).

4.5. Discriminant analysis

The discriminant analysis established by Ertlen et al. (2010) was applied on the reference database. In order to reduce the size of the matrix, the spectra of 10,000 to 7,312 cm^{-1} and 4,032 to 4,000 cm^{-1} were removed as no relevant information can be extracted from these ranges (Vysloužilová et al. 2015), resulting in a matrix of 409 columns (wave bands) and 427. Canonical Variate Analysis (CVA) was then used on the data, performed using IBM SPSS Statistics software (version 25.0).

CVA is an established procedure (Viscarra Rossel and Webster 2011). The data are in a matrix Z (see above) with a total variance-covariance matrix T. This matrix is partitioned into two sub-matrices – one for grassland and one for woodland. These two sub-matrices have a pooled within-class variance-covariance matrix W and a between-class variance-covariance matrix B. The latent roots (eigenvalues) and vectors of the matrix $W^{-1}B$ are found using the following general formula:

$$|\mathbf{W}^{-1}\mathbf{B} - \lambda\ \mathbf{I}| = 0$$

where λ are roots and I is an identity matrix. As only two classes are used, there is a single latent root, λ. The solution to the equation

$$(\mathbf{B} - \lambda\mathbf{W})\,\mathbf{c} = 0$$

gives c, the cannonical vector, in this case also necessarily a single one. The cannonical scores of the soil samples are acquired using the following formula:

$$\mathbf{y} = \mathbf{z}^{\mathrm{T}}\mathbf{c}$$

The mean cannonical points can be obtained using the means of spectra by computing:

$$\bar{\mathbf{y}} = \bar{\mathbf{z}}^{\mathrm{T}}\mathbf{c}$$

The Mahalanobis distance (D) between the classes is calculated from:

$$D^2 = \mathbf{d}^{\mathrm{T}}\mathbf{W}^{-1}\mathbf{d}$$

where d is the vector of differences between the means of the two classes (Ertlen et al. 2010).

The number of variates plus the number of groups must be less than the number of individuals, hence why the size of the data matrix was reduced (Vysloužilová et al. 2015)

The second step involved the application of the discriminant function calculated from the reference library on the unknown spectra obtained from the Minnesota sites. The comparison of values of these samples enables the evaluation of the origin of the soil organic matter (Vysloužilová et al. 2015).

4.5.1. Stable isotopes

The samples were pretreated and analyzed in the United States by Mason, Kasmerchak et al. (2018) of the Department of Geography at the University of Wisconsin–Madison. 10 g of each (untreated) sample were dried at 100 °C, pulverized, and treated with 2N hydrochloric acid for at least 16 hours at a temperature of 23 °C in order to remove carbonates. After the removal of carbonates, the samples were dried at 100 °C and pulverized for a second time. Following pretreatment, the samples were analyzed using a dual-inlet mass spectrometer with continuous capabilities coupled with an autosampler (Feggestad et al. 2004).

The relative abundance of ^{13}C in soil organic matter is represented as $\delta^{13}C$, the difference between the isotopic ratios of the sample and the standard, Vienna Pee Dee Belemnite (VPDB; Smith and Epstein 1971) in per mille using the following equation:

$$R = \frac{^{13}C}{^{12}C}$$

$$\delta(‰) = \left[\frac{R_{sample} - R_{RDB}}{R_{PDB}}\right] \times 1000$$

where R_{VPDB} = 0.0112372 (Ehleringer and Cerling 2002 in Feggestad et al. 2004).

5. Results

5.1. Near-infrared spectroscopy

The discriminant function was applied first applied on the reference database of 427 samples with known vegetation history. This way, two groups of soils were distinguished: under grassland and under woodland. 135 samples were classified as forest soils, the scores of the discriminant function ranging from to 2.84 to 9.58 (fig. 12). 292 samples were classified as grassland soils, the scores ranging from -4.81 to 0.39 (fig. 12). The classification was 100% accurate. For the reference library, the discriminant score for soils under grassland averages -2.59

while the average score for soils under forest is 5.60. The Mahalanobis distance, that is the distance between these two values, is thus 8.19 (fig. 12).

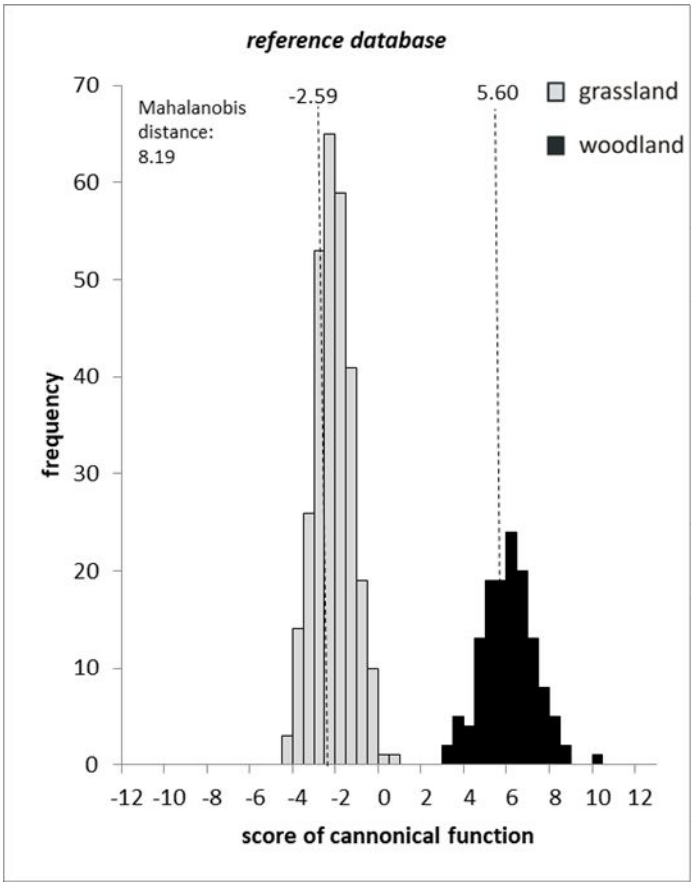


Figure 12 Histogram of cannonical function scores for the reference library.

Out of the 35 obtained samples from Minnesota, it was possible to use only 34 due to technical difficulties with obtaining spectra from one sample (site W-22). The discriminant function was then applied on this set. Of these 34 samples, only two (from two different sites) were classified as belonging to the group of soils under forest using the discriminant function: W-151 and P-98_4 (tab. 5). The rest were classified as grassland. All samples were classified successfully with no samples outside of these two defined groups.

In the set of 34 studied samples, the discriminant function score mean for soils classified as grassland is -2.67, with values ranging from -6.16 to 1.05, while the mean for forest soils is 6.40 with values of 1.67 and 11.12 (fig. 13). If this set is limited to Chernozem-like soils (USDA Mollisols), totaling 24 samples from 6 sites (tab. 14), the mean function score is -3.07 (scores ranging from -6.16 to -0.01) for grassland soils while the single Chernozem-like sample classified as woodland in this analysis has a score of 11.12 (fig. 14).

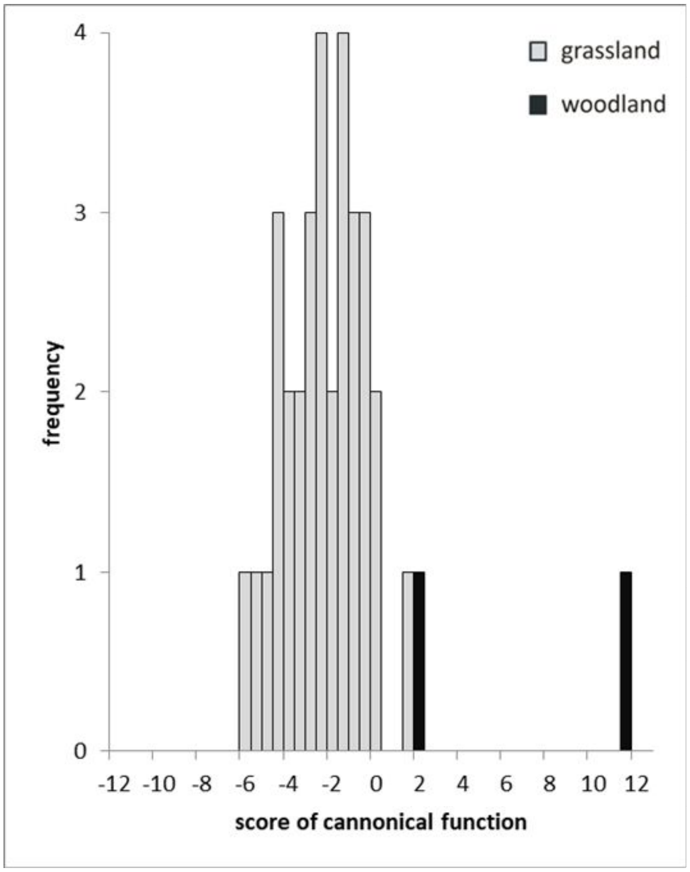


Figure 13: Histogram cannonica lfunction scores for the Minnesota sites.

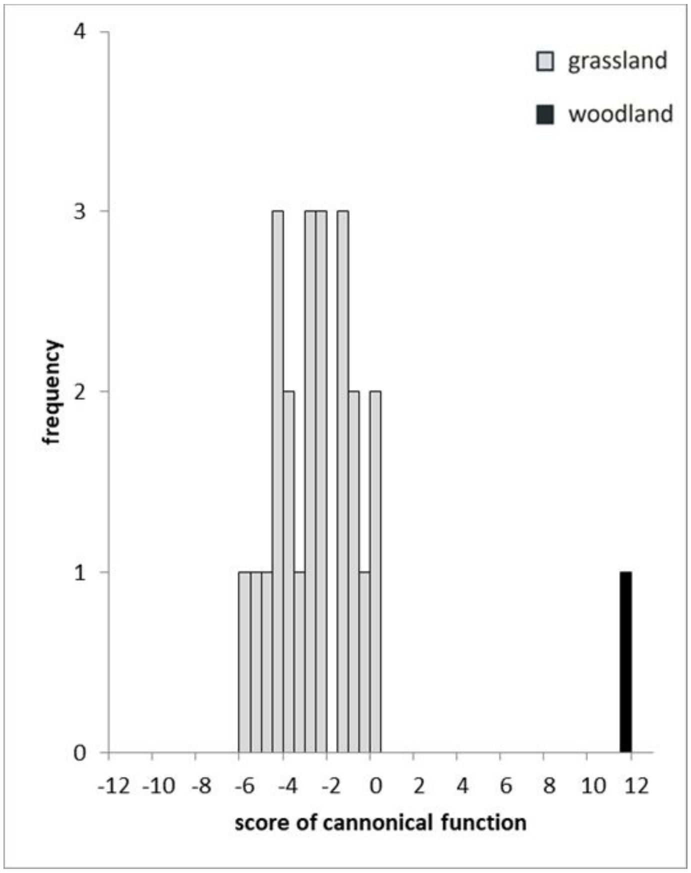


Figure 14 Histogram of cannonical function scores for Chernozem-like soils.

~~5.1.~~5.2. δ^{13}C

The δ^{13}C measurements were performed by Kasmerchak, Mason et al. (2018). A total of 34 samples, corresponding to those near-infrared spectroscopy analyses were successfully performed on (see above). To simplify the division into four groups by Kasmerchak et al. (2018) described in previous chapter, the threshold of -22‰ (Boutton 1996) was used to divide the samples into two groups: forest (with values of -22‰ and less) with SOM originating in the vast majority from C3 plants and grassland (more than -22‰) where a marked influence of C4 plants on the composition of soil organic matter is suspected. Values in the typical range of C4 dominated environments cannot be expected in the region, given the species composition of grassland ecosystems.

Of the 34 total samples, 20 were classified as forest with a mean $\delta^{13}C$ value of -25.7‰ and a minimum of -28.33‰, while 14 were classified as grassland soils, averaging -20.8‰ (fig. 15) and a maximum of -18.58‰. If the set is limited to the 24 samples from Chernozem-like soils (USDA Mollisols), 10 samples are classified as forest soils with an average value of -24.1‰ and a minimum of -26.35‰. 14 samples are classified as grassland soils and are identical to the 14 samples in the larger set, thus averaging -20.8‰ VPDB (fig. 16) with a maximum of -18.58‰. Of the two samples labeled as forest using the discriminant function on NIRS data, only one (W-151) can be classified as such using $\delta^{13}C$. The other sample, P-98_4, shows a below average value of $\delta^{13}C$ for grassland soils (tab. 5).

Due to the low percentage of C4 species found throughout Europe, $\delta^{13}C$ analysis was not performed on any of the samples used by Vysloužilová et al. (2014) to create the reference database.

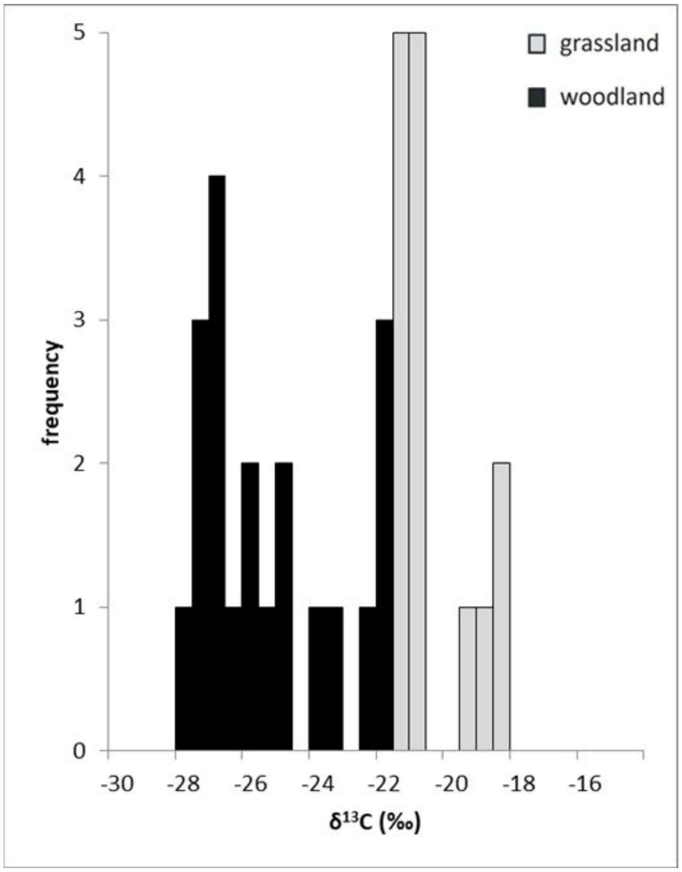


Figure 15 Histogram of $\delta^{13}C$ values for the Minnesota sites

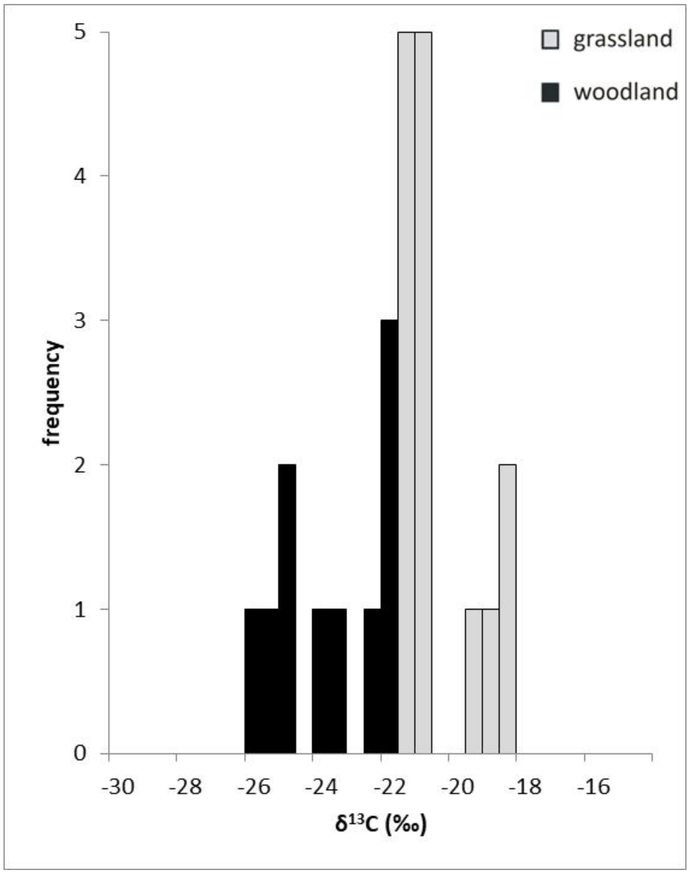


Figure 16: Histogram of $\delta^{13}C$ values for Chernozem-like soils.

~~5.2.~~5.3. Statsistical analysis

Given the nature of the data (non-normal distribution), Spearman's rank correlation coefficient was used on various data subsets, based on which t-values were calculated.

Using a two-tailed test, p-values were then used to determine the significance of correlations while critical t-values (at 0.05 significance level) were also calculated to confirm the significance.

The correlation of the scores of cannonical function obtained from NIRS data and δ^{13}C was first tested on the full dataset of 34 samples (or pairs of data for the purpose of testing), as lower scores of cannonical function and higher δ^{13}C values were found in grassland soils while lower higher scores of the function and lower δ^{13}C values appear to be typical of forest soils. However, no significant correlation was found with a p-value of 0.154 (fig. 17, tab. 4).

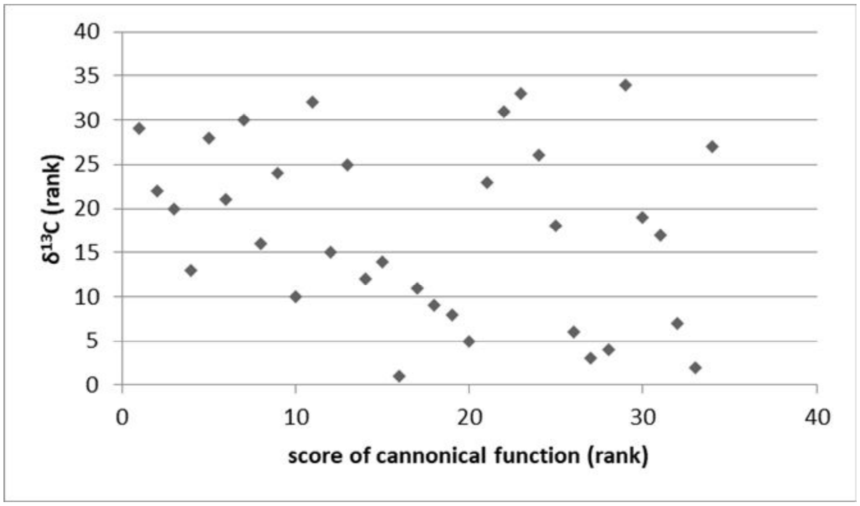


Figure 17: Relation of scores of cannonical function and δ13C (ranks).

The same was then tested on subset made up of 10 Luvisol (USDA Alfisol) samples. Again, no significant correlation was discovered, the p-value being equal to 0.229 (fig. 18, tab. 4).

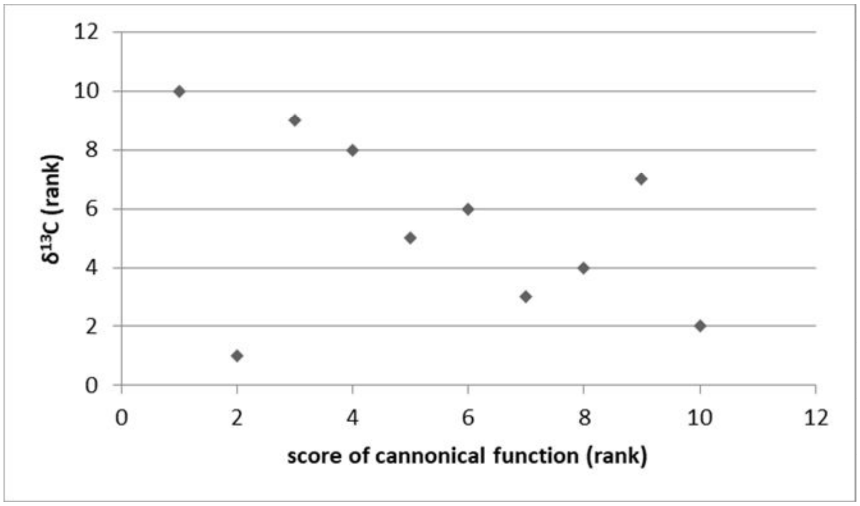


Figure 18: Relation of scores of cannonical function and δ13C (ranks); Luvisols.

A test of the correlation of the scores of cannonical function obtained from NIRS data and $\delta^{13}C$ was then performed on a subset of 24 samples collected at sites representing Chernozem-like (USDA Mollisol) soils. No significant correlation was found, $p = 0.774$ (fig. 19, tab. 4). The correlation of the scores of the discriminant function (NIRS data) and the depth at which the sample was collected was then put to test. This was made possible by the fact that the Chernozem-like sites were sampled at various depths, unlike the Luvisols of which only topsoils samples were available. A p value of 0.010 was calculated in this case, thus the null hypothesis of no correlation between depth and scores of cannonical function (NIRS) was rejected (fig. 20, 22, tab. 4). The correlation of the $\delta^{13}C$ values and depth was also tested. In this case, the p value reached 0.001, hinting at a significant correlation between these two variables (fig. 21, 23, tab. 4).

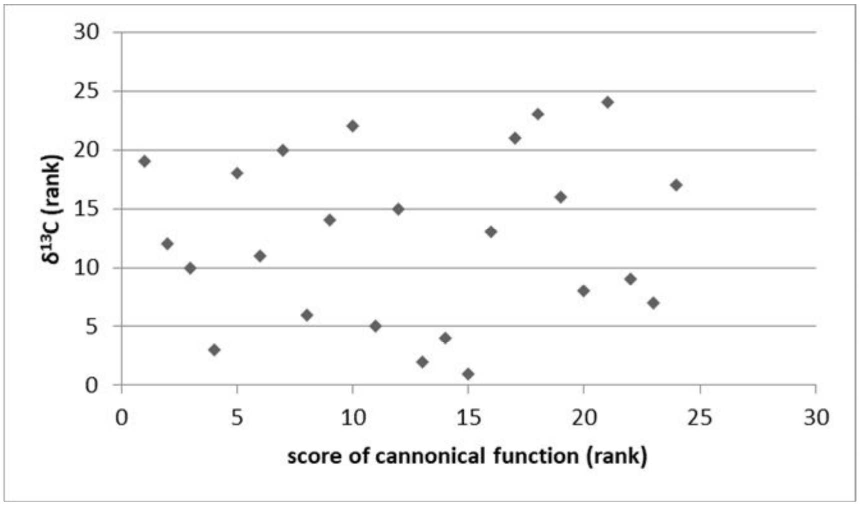


Figure 19: Relation of scores of cannonical function and δ13C (ranks); Chernozem-like soils.

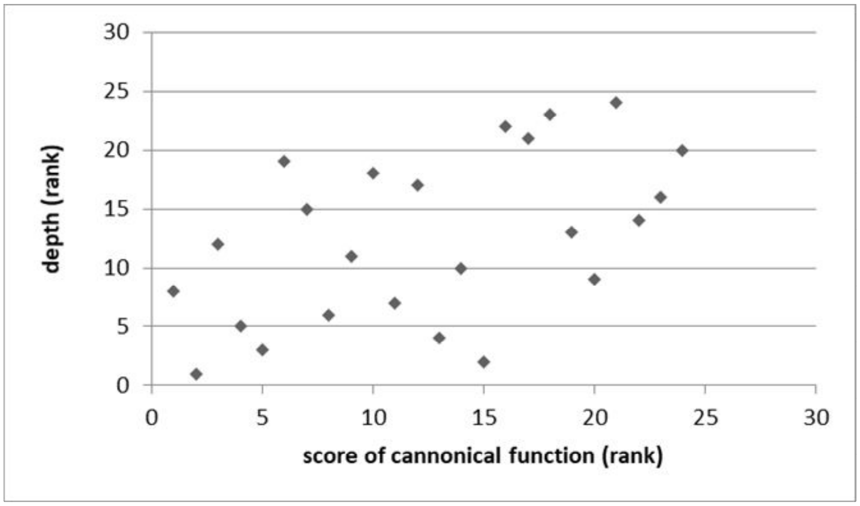


Figure 20: Relation of scores of cannonical function and depth (ranks); Chernozem-like soils.

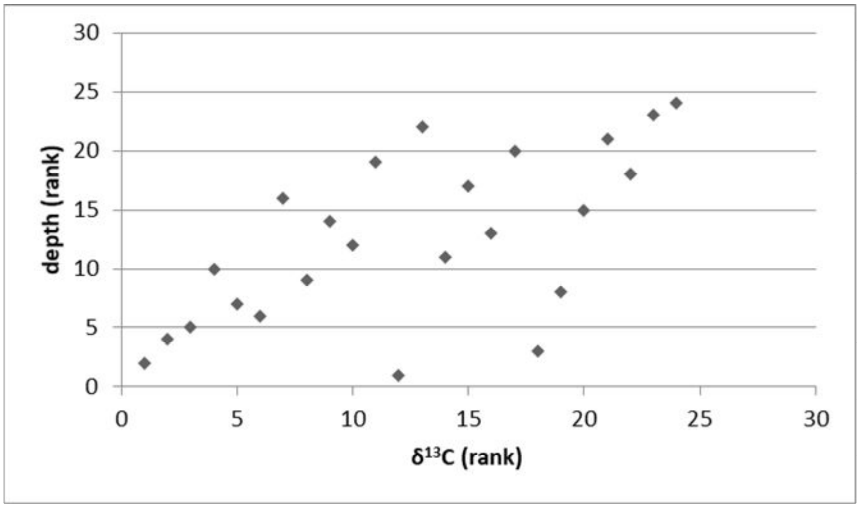


Figure 21: Relation of δ13C and depth (ranks); Chernozem-like soils.

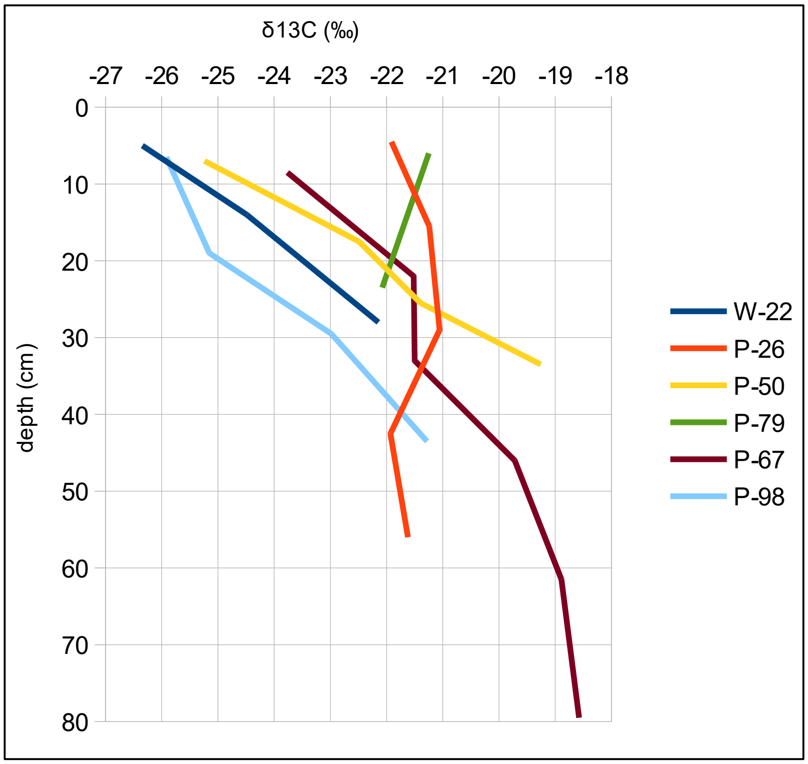


Figure 23: Relation of δ13C and depth, Chernozem-like soils.

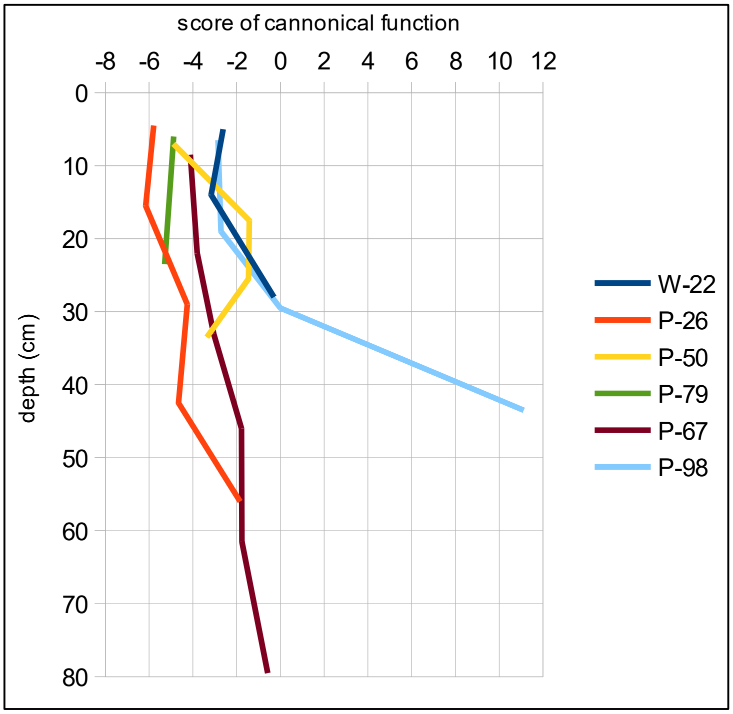


Figure 22: Relation of scores of cannonical function and depth; Chernozem-like soils.

Finally, the correlation between δ^{13}C values and scores of cannonical function (obtained from NIRS data) was tested on the Chernozem-like samples other than the topsoils, totaling 18 samples. No correlation was detected with a p value of more than 0.9 (fig. 24, tab. 4).

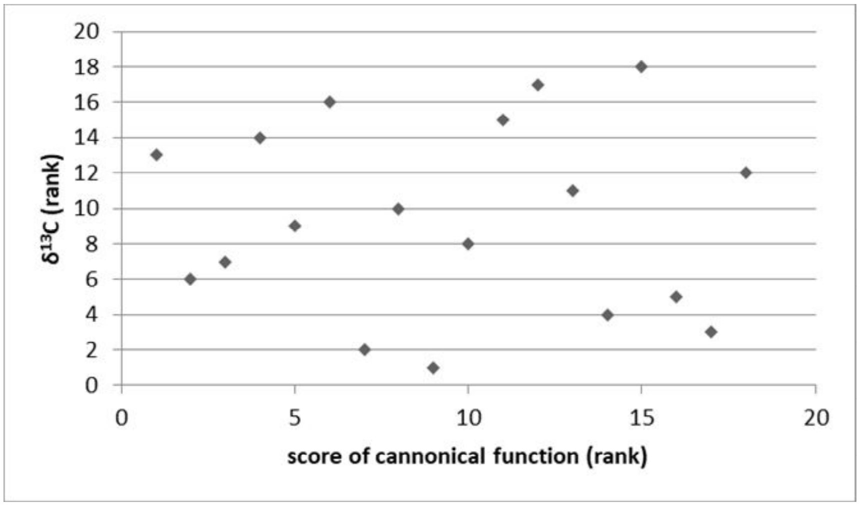


Figure 24: Relation of δ13C and depth (ranks); Chernozem-like soils without topsoil.

all samples			
null hypothesis	*result*	*p-value*	*number of samples*
no correlation between δ^{13}C values and scores of cannonical function (NIRS)	not rejected	0.154	34
Luvisols (USDA Alfisols)			
null hypothesis	*result*	*p-value*	*number of samples*
no correlation between δ^{13}C values and scores of cannonical function (NIRS)	not rejected	0.229	10
Chernozem-like soils (USDA Mollisols)			
null hypothesis	*result*	*p-value*	*number of samples*
no correlation between δ^{13}C values and scores of cannonical function (NIRS)	not rejected	0.774	24
no correlation between depth and scores of cannonical function (NIRS)	**REJECTED**	0.01	24
no correlation between depth and δ^{13}C values	**REJECTED**	0.001	24
Chernozem-like soils (USDA Mollisols) without topmost samples			
null hypothesis	*result*	*p-value*	*number of samples*
no correlation between δ^{13}C values and scores of cannonical function (NIRS)	not rejected	0.913	18

Table 4: Results of the statistical analysis.

sample	score NIRS	$\delta^{13}C$ (‰)	veg. NIRS	veg. $\delta^{13}C$	mean depth (cm)	soil (USDA)
E133	-0.85	-27.61	G	W	8.5	A
E215	-1.31	-27.27	G	W	6.5	A
E27	1.05	-27.14	G	W	5.0	A
E73	-2.70	-28.33	G	W	7.5	A
W151	1.67	-27.73	W	W	5.0	A
W171	-3.68	-26.50	G	W	5.0	A
W238	-2.24	-27.10	G	W	6.5	A
W39	-0.60	-27.59	G	W	5.5	A
W44	-2.44	-26.75	G	W	6.0	A
W70	-1.95	-27.30	G	W	6.0	A
P26_1	-5.79	-21.91	G	G	4.5	M
P26_2	-6.16	-21.24	G	G	15.5	M
P26_3	-4.25	-21.06	G	G	29.0	M
P26_4	-4.66	-21.93	G	G	42.5	M
P26_5	-1.84	-21.62	G	G	56.0	M
P50_1	-4.89	-25.24	G	W	7.0	M
P50_2	-1.42	-22.49	G	W	17.5	M
P50_3	-1.46	-21.40	G	G	25.5	M
P50_4	-3.36	-19.26	G	G	33.5	M
P67_1	-4.11	-23.76	G	W	8.5	M
P67_2	-3.80	-21.52	G	G	22.0	M
P67_3	-3.05	-21.50	G	G	33.0	M
P67_4	-1.78	-19.72	G	G	46.0	M
P67_5	-1.76	-18.89	G	G	61.5	M
P67_6	-0.58	-18.58	G	G	79.5	M
P79_1	-4.88	-21.25	G	G	6.0	M
P79_2	-5.29	-22.08	G	W	23.5	M
P98_1	-2.85	-25.92	G	W	6.5	M
P98_2	-2.71	-25.15	G	W	19.0	M
P98_3	0.00	-22.99	G	W	29.5	M
P98_4	11.12	-21.28	W	G	43.5	M
W22_1	-2.61	-26.35	G	W	5.0	M
W22_2	-3.16	-24.49	G	W	14.0	M
W22_3	-0.29	-22.15	G	W	28.0	M

Table 5: Measured values and properties of studied samples.

6. Discussion

6.1. Near-infrared spectroscopy

Despite various sources of information to the contrary (Kasmerchak et al. 2018), only two samples were identified using an analysis of NIRS spectra as being from soils under forest. While some discrepancy between various methods of determining past vegetation is to be expected, it is fair to say that the use of near-infrared spectroscopy in the studied region using a methodology and reference library devised on a different continent proved unsuccessful.

The methodology was previously successfully applied on Chernozem-like soils in Europe by Vysloužilová et al. (2015) and on wider variety of soils (using different reference databases) by a number of other authors (Ertlen et al. 2010, 2015; Viscarra Rossel and Webster 2008). The reference database used in this thesis was nearly identical to the one used by Vysloužilová et al. (2015). Its extent was purposely chosen to be made up of soils on loamy calcareous materials, similar in characteristics to the parent material found in the study area. While the origin of the material is not necessarily the same, the studied soils themselves are of similar morphologies.

There are two main reasons that may contribute to the stark differences between the results of different methods. One of them is climate. The reference database covers locations in Central and Eastern Europe where, perhaps with the exception of the easternmost sites, the continentality of the climate in Minnesota is difficult to replicate. The climate differences have the potential to cause differences in the composition (and decomposition) of the soil organic matter (Brady and Weil 2008). This would result in differing NIRS signatures.

Another factor is vegetation. In both regions, a large percentage of plants is of related or even identical genera (Pyankov et al. 2010, Teeri and Stowe 1976). However, it is difficult to estimate any effect this might or might not have on the composition of the soil organic matter that would affect NIRS fingerprints.

Still, the idea of using a NIRS reference database on a more global scale without the need of collecting hundreds or thousands of samples over millions of square kilometers is worth investigating further. A better match of climatic conditions would seem to be one of the possible ways of overcoming the discrepancies. Therefore, investigating the feasibility of using a reference database with more focus on Eastern Europe would seem like an effort worth pursuing without being prohibitively costly for researchers based in Central Europe.

The observed general trend of the NIRS spectra-derived function scores increasing with depth in the Chernozem-like profiles is not entirely inconsistent with the vegetation history suggested by Kasmerchak et al. (2018) as the two sites with the largest recorded increase in scores are currently observed to be under forest, making the existence of soil organic matter of woodland origin in the profiles plausible.

6.2. Stable isotopes

The $\delta^{13}C$ values representing abundance of C3 and C4 plant material in the soil organic matter (Boutton 1996) are largely consistent with the vegetation history of the sites as described by Kasmerchak et al. (2018) using 19th century public land surveys and other sources.

The trend of $\delta^{13}C$ values increasing with depth in the Chernozem-like profiles has a possible explanation in the current vegetation, known to be non-native C3 grasses at most sites with the rest currently under forest, also a C3 plant dominated environment (Boutton 1996, Kasmerchak et al. 2018). The majority of sites are believed to have been under grassland throughout the Holocene until the turn of the 20th century (Kasmerchak et al. 2018). The increase in values appears to be consistent with a higher percentage of C4 plants contributing to the composition of the soil organic matter in the past, making the past presence of native grassland cover plausible as a considerable percentage of grass species native to the region are known to utilize the C4 metabolic pathway (Teeri and Stowe 1976). Thus, the Chernozem-like soils in the region can be characterized as grassland or prairie soils.

6.3. General

The inability of using δ^{13}C as a reliable method to study past vegetation throughout Europe due to the very low percentage of C4 plant species (Pyankov et al. 2010) restricts the possibilities of recording the vegetation history of Chernozems and Chernozem-like soils in the region on a large scale, which could serve as a valuable contribution to the ongoing discussion on the origin of Chernozems and their vegetation history (Eckmeier et al. 2007, Lorz and Saile 2011, Suchodoletz et al. 2017, Vysloužilová et al. 2014; and others). Numerous authors have rejected the idea of Chernozems as purely grassland or steppe soils in many parts of Europe (Eckmeier et al. 2007, Lorz and Saile 2011, Vysloužilová et al. 2014; and others). The emergence of modern and relatively low-cost methods such as near-infrared spectroscopy has the potential to at least partially compensate for the lack of information on vegetation history available in other regions using more traditional methods such as stable isotopes (Ertlen et al. 2015).

7. Conclusion

The attempt at using a reference library from a geographically distant location to extract data using near-infrared spectroscopy at new Chernozem-like sites in Minnesota, USA was not succesful. The use of a NIRS database outside of its immediate geographical setting seems to remain limited for now. The findings do however provide data and information that can be used to build on in the future to try and improve the accuracy of reference databases for their use on a more global scale.

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