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What do they seek for? Functional link of plant-environment interactions to foraging ecology of large herbivores

Bachelor thesis

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Declaration

I hereby declare that this thesis entitled: What do they seek for? Functional link of plant-environment interactions to foraging ecology of large herbivores, is my own work and all the sources have been quoted and acknowledged by means of complete references.

In Prague date

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Abstrakt

Cílem této bakalářské práce bylo zjistit, zdali existuje funkční vztah mezi obsahem makroprvků v půdě a v trusu zubra evropského (*Bison bonasus*). Jednotlivými dílčími cíli bylo stanovit: 1) funkční vztah mezi koncentrací celkového N v půdě a v trusu zubra evropského 2) funkční vztah mezi koncentrací rostlinám dostupného P v půdě a celkového P v trusu zubra evropského 3) vzájemné vztahy mezi koncentracemi makroprvků (N, P, Ca, Mg, K) v trusu zubra evropského 4) rozdíl v potravě zubra evropského mezi letním a zimním obdobím v chovné stanici Cherga. Počáteční hypotézou bylo, že i když mezi půdou a rostlinou existuje přímý funkční vztah, tj. živiny v rostlině do jisté míry reflektují obsah dostupných živin v půdě, tento funkční vztah nebude platit na úrovni půda- trus, jelikož si velcí býložravci dokáží případný nutriční nedostatek vykompenzovat potravními strategiemi. Sběr vzorků půdy a trusu probíhal ve třech vzdálených populacích zubra evropského: v České republice v oboře Židlov, v chovné stanici Cherga v ruském Altaji a v Nizozemí v národním parku Kraansvlak, čítající dohromady 40 vzorků trusu a 12 vzorků půdy. Vzorky trusu byly testovány na obsah koncentrací N, P, K, Ca, Mg, NDF ADF, ADL a vzorky půd na obsah koncentrací celkového N a rostlinám dostupných P, K, Ca, Mg, vyjádřených v 100% sušině , navíc byly stanoveny hodnoty pH a $C_{org.}$.

Na základě výsledků bylo stanoveno, že mezi koncentrací N v trusu a v půdě byl přímý funkční vztah ($P= 0.009$, $r= 0.57$), nicméně mezi koncentrací P v trusu a rostlinám dostupným P v půdě nebyl nalezen žádný funkční vztah ($P= 0.35$, $r= -0.22$). Dále bylo zjištěno, že lokality mají výrazný vliv na koncentraci makroprvků v trusu zubra evropského. Mezi potravní nabídkou zubra evropského v létě a v zimě v chovné stanici Cherga existoval významný rozdíl ($P< 0.001$), s významně vyšší koncentrací makroprvků (N, P, K, Ca, Mg) v trusu z letního období.

K potvrzení nebo vyvrácení případného funkčního vztahu mezi obsahem živin v půdě a v trusu velkých býložravců je zapotřebí dalších výzkumů a materiálů.

Klíčová slova: potravní strategie, velcí býložravci dusík, fosfor, interakce půda-rostlina

Abstract

The aim of this bachelor thesis was to conduct the investigation of the relation between macronutrients in soil and faeces of European bison (*Bison bonasus*). Particular aims were to determine: 1) functional relationship between concentrations of total N in soil and in faeces of European bison 2) functional relationship between concentrations of plant available P in soil and total P in faeces of European bison 3) mutual relationships of concentration of macronutrients (N, P, Ca, Mg, K) in faeces of European bison 4) the difference in diet quality of European bison between summer and winter period in Cherga breeding station. The initial hypothesis was that despite of the fact that nutrients in plant are in direct proportion with soil accessible nutrients, this relation between soil and faeces will not work directly, since herbivores can balance potential nutrient deficiency through foraging strategies. Sampling of soil and faeces was done in three distant populations of European bison: in the Czech Republic in Židlov, in Russian Altai mountains in Cherga and in the Netherlands in Kraansvlak, comprising together 40 faecal and 12 soil samples. Faeces samples were tested on concentration of N, P, K, Ca, Mg, NDF ADF, ADL and soil samples on concentration of total N and plant available P, K, Ca, Mg expressed in 100% of DM, further the pH value and C_{org} were determined.

On the basis of the result, it can be concluded that there was direct functional link between faecal N of European bison and total N in soil ($P = 0.009$, $r = 0.57$), however no relationship between faecal P and plant available P in soil was found ($P = 0.35$, $r = -0.22$). Furthermore, it was proved that habitats play important role in content of macronutrients in faeces of European bison. Finally, there was marked difference ($P < 0.001$) between diet quality in Cherga summer/ winter period, with significantly higher macronutrients concentrations (N, P, K, Ca, Mg) in faeces European bison from summer period.

In conclusion, more investigation is needed to be done, in order to support or disprove any relation between nutrients in faeces of large herbivores and in soil in their habitats.

Key words: foraging strategies, large herbivores, nitrogen, phosphorus, plant-environment interactions

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List of the contractions used in the thesis

ADF (acid-detergent fibre)
ADL (acid-detergent lignin)
Al (aluminium)
As (arsenic)
B (boron)
C (carbon)
Ca (calcium)
Cd (cadmium)
Cl (chlorine)
Co (cobalt)
CO_2 (carbon dioxide)
C_{org} (organic carbon)
Cr (chromium)
Cu (copper)
DM (dry matter)
F (fluorine)
Fe (iron)
FN, FP (faecal nitrogen/ phosphorus)
H (hydrogen)
H_2O (water)
$H_2PO_4^-$, HPO_4^{2-} (dihydrogen phosphate ion, monohydrogen phosphate)
H_2S (hydrogen sulfide)
H_3BO_3 (boric acid)
I (iodine)
K (potassium)
Mg (magnesium)
Mn (manganese)
Mo (molybdenum)
MoO_4^{2-} (molybdate)
N (nitrogen)
Na (sodium)
NDF (neutral-detergent fibre)
NH_3, NH_4 (ammonia, ammonium)
Ni (nickel)
NO, N_2O (nitrogen oxide, nitrous oxide)
O (oxygen)
P (phosphorus)
S (sulphur)
Se (selenium)
Si (silicon)
SO_2 (sulphur dioxide)
SO_4^{2-} (sulfate)
Zn (zinc)

1 Introduction

The principal and fundamental link between soil, plants and large herbivorous animal could be provided by two of the most essential nutrients nitrogen (N) and phosphorus (P). Phosphorus (P) together with nitrogen (N), are the essential elements in ecosystem functioning and dynamics linking soil biology with plant and animal physiology, ecology and behaviour. P and N are nutrients the most frequently limiting primary productivity in terrestrial ecosystems (Ngai and Jefferies, 2004; Hillel et al. 2005; Elser et al., 2007; Vitousek et al., 2007). Nowadays, it is already known that there is direct functional link between soil- plant interactions. In general terms it means that the plants nutrition is reliant on availability of nutrients in soil, so content of nutrients in plants is in direct proportion with content of accessible nutrients in soil (Chadwick et al., 1999; Augustine et al., 2003; Elser et al., 2010). Thus, the different types of soils provide less or more nutrients support of macro and trace elements for plants growth, e.g. the area of ancient landscape is typical of rock derived nutrients (e.g. P) impoverished soils to the contrary of young fertile soils which provide great amount of rock derived accessible nutrients for plants (but are limited by N) (Lambers et al., 2010).

Wild herbivores are primary dependent on the quality of grazing land and plants.

Nitrogen foliar content has been widely considered as a key factor of plant – animal interactions (Mattson, 1980). On the other hand, phosphorus requirements are more tightly linked to animal fitness. The lack and unavailability of mineral and other nutrients may limit population of herbivores in some areas. It was proved that the habitat with poorer occurrence of several nutrients has a negative impact on animal's health, physical condition and reproduction as well. For example, the habitats in the ancient landscapes with deficiency of phosphorus (P) cause reduction of nutrients in the body of lactating females and restrict the process of lactation and as a consequence of this process mortality of offspring rapidly increased (Freeland and Choquenot 1990).

However, there are a lot of surveys and information focused on soil, plant and animal interactions, this topic is still current because linkages between nutrients flow in soil, plant and final consumer- large herbivores are highly complex and they are rarely studied in an ecosystem context. Furthermore, for only a few wild herbivores species the details of their dietary mineral requirement are known. Due to this, more investigation is needed to be done in accordance with conservation of plant diversity as an essential factor for balanced nutrition and nutrient requirements of wild herbivores.

2 Literature review

2.1 Phosphorus and nitrogen

2.1.1 Role in plant and animal metabolism

Phosphorus and nitrogen are the most widespread limiting nutrients in plants as well as in animal's growth and development, due to their large requirement and the finite ability of soils supplying them in sufficient accessible forms.

Nitrogen and phosphorus are the cornerstones (foundation stone) of many essential biochemical molecules vital to metabolism function in both plants and animals organism.

Organic N is participating on structure of proteins as amino acid (e.g., glutamine, glycine, lysine etc.), amino sugar (e.g., glucosamine, galactosamine), nucleosides (adenine, guanine, thymine, uracil, cytosine), peptides, phospholipids (e.g., phosphatidylserine), vitamins (e.g., niacin), creatine, cyanide, allantoin, alkyl amines and urea. Phosphorus is primary component of energy molecules ATP (adenosine triphosphate), ADP, AMP, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), phospholipids, coenzyme phosphopyridine nucleotides ($NADP^+$), phytin (P storage molecules mainly in seed-inositol hexaphosphate) and component of some intermediate product (e.g. glycolysis, glycolysis) (Hillel et al., 2005).

Phosphorus and nitrogen are both individually or in combination the most common limiting elements in the Earth's ecosystems (Vitousek et al., 2010). There was a long-lasting belief about the different demands of different ecosystems on the various limiting elements. In accordance with these old paradigms, N was stated as the primary limiting element in terrestrial and marine ecosystems and P as main limiting element in freshwater ecosystems. Nevertheless, the latter-day research results advert to uniformity of autotroph response to N and P enrichments with pointing to equivalence of N and P limitation. Furthermore, joint N and P enrichment indicate comparably strong synergistic effect in all noted ecosystems, hence N and P limitation seem to have equal importance in terrestrial and freshwater ecosystems (Elser et al., 2007).

N and P have very strong connexion and influence on each other, i.e. they can have stimulation as well as constraining effects on each other, e.g. particularly the excess of nitrogen can strongly support the uptake of phosphorus and other nutrients by plant, due to its boosting effect on biomass production (Elser, 2007).

2.1.2 Resources of P and N

As the Walkers and Syers (1976), summarized by Vitousek et al. (2010), suggested phosphorus soil availability comes from rock weathering, thus in this hypothesis there have been a fixed stock of P amount in the ecosystems outset formation and as a consequence of this condition even the infinitesimal losses cannot be easily replenished. Due to this scientific knowledge, it is known that ecosystems with very old soils, i.e. majority of tropical soils, which are deeply weathered and infertile such as in Cape Region in South Africa or in South- Western Australia (Lambers et al., 2010), can become P depleted and if this stuff will continue it will be followed by final total P depletion, named by Walkers and Syers “terminal steady state”.

The over-riding role of phosphorus in soils play primary minerals, which provide storage function of vast majority of P. Primary P minerals are namely apatites, which are highly insoluble Ca- phosphate compound with general formula $Ca_{10} (PO_4)_6X_2$, where X can be represented by F^-, OH^-, Cl^-. The P is gradually released from this mineral either by weathering or by microbial and/ or plant roots activity.

On the contrary, N is collected from the atmosphere either quicker via biological fixation by symbiotic N- fixer organism or non-symbiotic free living N- fixer prokaryotic bacteria, working on the presumption that N fixer organism dominate in the early ecosystem development, or via physically- chemical atmospheric dry and/or wet deposition, which is slower and occur undoubtedly in smaller scale than biological fixation. However, nowadays due to raised global pollution mainly by anthropogenic activities, there is constantly increasing trend of obtaining N by acid rain contained nitric acid (HNO_3), formed in atmosphere by reaction of gasiform nitrogen, oxygen and water, which is not negligible as well. Therefore, in accordance with, above mentioned biological processes of N cycling, the young soils in many ecosystems may be rather N than P limited (Vitousek et al., 2010).

2.1.3 The nitrogen cycle in the environment

Nitrogen cycle is closely connected with C transformation and they play an irreplaceable role in all living systems in The Earth. The total amount of N on the Earth is suggested to be $1.68*10^{17}$t (Tlustoš et al., 2007) (see Table 1) from which is the highest amount contained in lithosphere accounting for 98% ($1.64*10^{17}$t) of total nitrogen. However, this nitrogen in form of igneous rocks, sediments and coal do not actively participate in environment N cycling due to high stability of N bond in these compounds (Hillel et al., 2005). Remaining 2% of total N is contained in atmosphere and only small part of residual N is in hydrosphere and biosphere. The majority of nitrogen in soil is present in organic forms.

Table 1 The total and plant accessible amount of N in the main Earth's constituents (Tlustoš et al., 2007)

Bodies	Total N		Accessible N	
	t N	%	t N	%
Lithosphere	$1.64*10^{17}$	97.63	$4.50*10^{14}$	10.39
Atmosphere	$3.86*10^{15}$	2.30	$3.86*10^{15}$	89.07
Hydrosphere	$2.30*10^{13}$	0.01	$2.30*10^{13}$	0.53
Biosphere	$2.8*10^{11}$	0.0001	$4.6*10^{11}$	0.01
Total sum	$1.68*10^{17}$	100.00	$4.3*10^{15}$	100.00

The most concentration of lithospheric N is situated in the upper layer (10-15 cm) of soil. For the reason of strong bonded N in majority compounds, only 2.5% of total N is in accessible form for plants and microorganisms nutrition (Hillel et al., 2005).

The major processes and transformation of N in soils can be divided into five pathways: mineralization (also ammonification), assimilation, nitrification, denitrification and N_2 fixation (Hillel et al., 2005). All these processes are strongly influenced by C:N ratio as well as C quantity and forms in soil, oxidation-reduction potential, soil moisture, temperature and aeration. As mentioned above (see resources of P and N), the inputs of N to soil cycle can be through symbiotic or non-symbiotic N_2 fixation, wet/dry deposition from atmosphere, decomposition of organic matter (animal and/or plant), organic (as well as urea, faeces) or inorganic fertilizers and slow releasing of N from rocks and other minerals. On the other hand, N loses from soil can be through: volatilization during denitrification (gaseous NO, N_2O, N_2) and mineralization (due the semi-finished product gaseous NH_3), immobilization- incorporation inorganic N into biomass and then plant harvesting, leaching N into water sources with possible eutrophication effect and erosion. Sometimes between N loses, not exactly from soil but as the accessible form for plant and microorganism uptake (NO_3^-, NH_4^+), can be included: immobilization especially NH_4^+ by some clays minerals (e.g. illite), thus making it constrained for plant use, or interreaction of inorganic N with soil organic matter with the same impact on plant utilization.

2.1.4 The soil phosphorus cycle

Total soil P ranges from 0.01 to 0.30% and contrary to N, majority of soil P is presented in mineral forms either in primary minerals (e.g. apatite group) or in secondary clay minerals, where makes insoluble compounds with Al, Fe and Ca. Phosphorus in soil occurs in three forms: Solution P (mainly as HPO_4^{2-} $H_2PO_4^-$, in smaller scale in organic forms), organic P (e.g. phosphate ester, phospholipids, nucleic acid, phytate) and inorganic P contained in primary or secondary minerals (see above) (Hillel et al., 2005).

The P cycle is cooperation between soil, plants and microorganisms. The over-riding processes in P cycle are represented by P uptake by plants and microbes, recycling of plant and animal residues, biological mineralization-immobilization transformation, fixation and/ or precipitation reaction at clay minerals and oxide surface and finally solubilization of P due to activity of microorganisms and/ or plants as well as through chemical reactions or soil mechanical disturbance (e.g. weathering, erosion) (Balík et al., 2008). Phosphorus losses can arise with erosion, run off (to surface water), leaching and harvest. Releasing of P is positively correlated with lower pH with optimum 6.5 (Hillel et al., 2005)

2.2 Nutrients resources in soil

The soil is fundamental constituent for all living terrestrial organisms. It is the upper layer of Earth's crust, formed in the process called pedogenesis, by weathering of parent material and activities of edaphic microorganisms during climatic influences and soil factors in the long period. The soil consists of gas soil phase (soil air), liquid phase (soil solution) and solid phase (mineral an organic). The soil solution consists of dissolved ions of chemical compounds, gass (especially CO_2) and some organic substances (particularly in rhizosphere) and provides the basic important source of available plant nutrients. At last, the solid phase of soil can be divided on mineral part (92-98%), which consists of primary aluminosilicate (feldspar, mica) and secondary aluminosilicate (illite, montmorillonite, kaolinite, allophane), and organic part (2-8%) formed by edaphic organism and inanimate organic materials including nonhumified organic substances (primary organic matter) and humic part (humic acid, fulvic acis and humic substances) (Hillel et al., 2005). The soil composition and fertility rely on both abiotic and biotic factors and the nutrients differ rapidly in ancient landscapes and in young landscapes (see below). And finally, soil provides essential nutrients as well as habitat to edaphic organisms, fungi and primary producers.

2.2.1 Different type of soil

There exist a lot of soil types on all over the world and the pedology discipline is focused on it. However, I will distinguish soil on two main groups: soil of ancient landscapes (OCBILs) and soil of young landscapes (YODFELs), according to Lambers et al. (2010). The abbreviation of OCBILs means: old, climatically buffered, infertile landscapes, and vice versa YODFELs mean: young, frequently disturbed, fertile landscapes. On this two different soil types diverse flora with various ecophysiological plants traits have been developed.

Ancient landscapes are characterized by nutrient impoverished soils, because they have not been glaciated or disturbed by other major natural catastrophic events (e.g. volcanic eruption) in recent time. Also their climate is more or less stable (due to buffering by oceans) with typical high biological diversity (Lambers et al., 2010). This soils, typical to tropics and subtropics area (e.g. Western Australia, South Africa), are represented by high amount of allophones, and sesquioxides. Allophanes are amorphous clay mineraloids, which have great capability to bind unbound nutrients (mainly P compound) in soil and building it in insoluble complex, and make it inaccessible to plants uptake (Hillel et al., 2005). These soils are usually P limited, or generally impoverished of rock derived minerals, not only for present of allophane but also for high stage of weathering corresponding to their age. The stock of rock derived nutrition which are getting loose by weathering, become depleted without replenishment, contrary to atmospherically derived elements are replenished continuously ($CO_{2,}$ N_2) (Chadwick et al., 1999).

On the other hand YODFELs are fertile soils of younger landscapes, i.e. Europe, where prevail N limitation to P limitation for the reason of relative young age of ecosystems (need of N assimilation). These soil were rejuvenated by glacial recession, volcanic eruption or other disturbances, initiating formation of new ecosystems and soils enriched of rock derived minerals supplementation with lack of atmosphere derived nutrients ($CO_{2,}$ N_2), where flora with N_2 fixing symbioses is predominating (Chadwick et al., 1999; Lambers et al., 2010).

2.2.2 Resources of minerals in soil

As mentioned above, the solid phase of soil consists of primary aluminosilicate and secondary aluminosilicate and together account for 92-98% of solid phase (Hillel et al., 2005). The primary aluminosilicate are rock forming minerals, providing main stock of mineral nutrients (e.g., P, K, Ca, Mg, Na, Fe, Al), which are released by weathering or by other disturbance to the soil. The nutrients are bound in feldspar minerals especially in orthoclase ($K(AlSi_3O_8)$) and plagioclase $Na(AlSi_3O_8)$, mica minerals- biotite (K, Mg, Fe) , olivine (Mg-Fe), amphibole, serpentine and talcum (Mg) and etc.

The main primary sources providing store of inorganic phosphorus are minerals encompassing phosphate group $(PO_4)^{3-}$, such as the most widespread apatite group of minerals, then for example turquoise minerals, monazite and vivianit.

However, primary minerals play the main role in storage and conservation of mineral nutrients in soil, they also make the bound nutrients inaccessible to direct plant nutrition at the same time. In contrary, the secondary aluminosilicate, consisted mainly from clay, play the direct role in plant nutrition and water sorption. Because they show high sorption capacity enabling to released ion of primary minerals being sorbed on the surface or in inside the secondary aluminosilicate, with subsequent releasing into soil solution by exchangeable and/ unexchangeable capacity or due to filling with water (releasing ion trapped inside). The secondary clay minerals as illite, montmorillonite, kaolinite and allophane consist of tetrahedron (Si:O) or octahedron (Al(Mg^{2+}, Fe^{2+}) : OH) strata (layers) in diverse ratio affecting the sorption capacity. The highest cation sorption has illite with 2:1 ratio, while highest anion sorption shows allophane. Ions released from this minerals are exploitable to plant and microorganism nutrition (Hillel et al., 2005).

2.3 Plant nutrients

2.3.1 Plant nutrition and requirement

Plant nutrient is term for those nutrients, which are necessary to plant metabolism, development and successful reproduction. These essential nutrients are divided by their concentration in plants to macronutrients and micronutrients. Macronutrients typically attain concentration >500 mg kg^{-1} in mature plants and are following: Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). Micronutrients attain concentration <100 mg kg^{-1} in mature plants and also their concentrations in soil are generally low (apart from Fe). Micronutrients are: Iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), boron (B), chlorine (Cl) and nickel (Ni). In the third class are useful elements: Sodium (Na), silicon (Si) and aluminium (Al), which are presented in plants, but their abundance and importance vary with plant species. The plant uptake the nutrients mainly from soil solution as cation or anoint, however some gasses nutrients (i.e. $CO_{2,}$ SO_2, H_2S) can be also absorbed by leaf stomata. The different forms of plant accessible nutrients and their functions are shown in Table 2.

Table 2 Forms of absorption and functions of essential nutrients in plants (Hillel et al., 2005)

Nutrient	Forms taken up by plants	Functions
Carbon	CO_2	Basic molecular component of carbohydrates, proteins, lipids, nucleic acid.
Hydrogen	H_2O	Central role in metabolism, importance in ionic balance as main reducing agent, key role in energy relation of cells.
Oxygen	CO_2 , O_2	Basic molecular component in all organic compounds.
Nitrogen	NH_4^+, NO_3^-	Important compounds ranging to nucleic acid to proteins.
Phosphorus	$H_2PO_4^-$, HPO_4^{2-}	Key role in energy transfer and protein metabolism.
Potassium	K^+,	Osmotic and ionic regulation, cofactor or activator of many enzymes.
Calcium	Ca^{2+}	Participation in cell division, maintenance of membrane integrity.
Magnesium	Mg^{2+}	Component of chlorophyll, cofactor for enzymatic reactions.
Sulphur	SO_4^{2-}	Similarity with phosphorus- participation of energetic reaction, part of some amino acid.
Iron	Fe^{2+}, Fe^{3+}	An essential component of heme and nonheme Fe emzymes and carries (cytochromes, ferredoxins), component of chlorophyll.
Zinc	Zn^{2+}	Essential component of some dehydrogenases, proteinases and peptidases, e.g. glutamic and malic dehydrogenases.
Manganese	Mn^{2+}	Involved in the O_2 evolving system of photosynthesis, component of arginase and phosphotransferase enzymes.
Copper	Cu^{2+}	Constituent of important oxidase-enzymes, e.g. cytochrome and ascorbic acid oxidase, lactase, importance in photosynthesis, protein and carbohydrate metabolism.
Boron	H_3BO_3	Activator of some dehydrogenase enzymes, essential for cell division and development, synthesis of cell walls components.
Molybdenum	MoO_4^{2-}	Component of nitrate reductase and N_2 fixation enzymes.
Chlorine	Cl^-	Essential for photosynthesis (splitting water) and enzymes activation, osmoregulation functions of plants growing in saline soils.

2.3.2 Plant uptake strategies

Plants have developed several strategies how to acquire hard accessible nutrients and thus maintain their metabolisms requirements. For example, due to common insufficient amount of accessible P in soils, plants have developed following strategies. Firstly, the roots have ability to secrete exudates, which can be represented by organic acids as citric acid, propanedioic acid, fumaric acid etc., and thus acidify the adjacent surrounding making alkaline compound more soluble. Secondly, they can secrete alkaline or acidic phosphatase in relation of purpose to lower or raise soil PH, and thus increase P availability. Thirdly, the capability of formation proteoid roots (cluster roots) is enhancing for roots nutrient uptake due to enlarged absorption surface. And finally, arbuscular mycorrhiza or ectomycorrhizal association is also very effective for widening the area from which can be nutrients collected, due to extension of roots by fungal mycelium by hypha in the soil, which should be better in competition with soil microbial organisms for example for P acquiring.

The special N acquiring strategy is symbiosis of some plant with N- fixing bacteria (e.g. legumes with rhizobia bacteria and some trees (e.g. alder, sea buckthorn) with actinomycete) which provide N in form of ammonia to plant, and get back energy in form of saccharide products (Hillel et al., 2005; Lambers et al. 2010).

2.3.3 Nutrients fluctuation in plants

Plants are characterized by marked mineral diversity, where mineral concentrations vary from different plant species to plant individual. Nutrient concentrations in plant material are primary influenced by mineral status of the soil in which it grows (“you are what you eat”) (Stapelberg et al., 2008). However, there are a range of other factor participating on plant nutrient fluctuation, such as the stage of plant maturity, genetic predisposition, environment and abiotic (weather) factors (Stapelberg et al., 2008, Ohlson and Staaland, 2001). Even, the different plant parts, organs and tissues differ from each other in mineral concentration. For example, most N and P is located in seeds (Mattson, 1980), majority of ash minerals (e.g. Ca, Mg, S) are found in leaves and abundance of potassium is found in inflorescence and juvenile plant parts/ organs. The young plants and/or plant parts are richer on mineral nutrients in comparison to old plants/ parts due to dilute effect. However, for wild herbivores is crucial, that the nutrients levels in plants is not stable, but depend on different season. Most of plants show seasonal trends in nutrients fluctuation, i.e. nutrient levels in plants are not constant but fluctuate during different seasons, with peak of highest nutrients concentration in spring and minimum in winter season, or with peak of wet season and minimum in dry season in the tropics and subtropics (Stapelberg et al., 2008). All these factors underlie for herbivores foraging strategies. Thus, it was concluded that plant species diversity is prerequisite to mineral diversity, which is essential to well-balanced diet for herbivores (Ohlson and Staaland, 2001). Ohlson and Staaland (2001) showed that aquatic plants have higher concentration of majority of elements than terrestrial plants, with exception of N, B and Mn, and can serve for example for moose as important mineral supplement.

2.3.4 Defensive mechanism of plants against large herbivores

Plants are endowed with variety of defensive mechanism, providing them some degree of protection against their consumer- herbivorous animals (Freeland et al., 1974). Plant can protect themself by physical avoidance, such as location, visibility, or by producing mechanical barriers, including spines, trichomes, tough cuticules, and/or by chemical avoidance through chemical substances, so-called plant secondary compounds, which are special for every plant (Danell (ed.), 2006). There is variety range of secondary compounds from simple organic compounds (e.g. nitrates, silicates) to alkaloids, terpenoids and glycosides, but the most important plant secondary compounds are tannins, especially condensed tannins. Tannins are phenolic defensive mechanism, present in greater amount mainly in shrubs, based on ability to precipitate plant proteins and gastrointestinal enzymes, resulting in reduced protein and cell digestion. Robbins et al. (1987) proved that tannins markedly reduced protein availability. However, there are various types of phenolics, which are part of more complex defensive mechanisms against large herbivores, which include gastrointestinal functions (e.g., decreased protein digestion and gut wall permeability) and internal cellular and organs effect. Thus, high intake of tannins can inhibit digestion of plant material, increase excretion of essential minerals from animal organism or even lead to physiological impairment, due to high toxicity. Nevertheless, despite of all negative effect of tannins, the wild herbivores have adapted on their adverse impact and can restrict and/or eliminate the negative effect of tannins by detoxification strategies, such as elevating gut pH and/or use of surfactants as well as by foraging strategies (Freeland et al. 1974; Robbins et al., 1987).

2.4 Animal nutrients

The quality of productivity, physical condition and fitness of large herbivores is in direct proportion with the nutritional quality of their diet. In the optimal condition, the ingested food should provide sufficient content of essential nutrients as well as digestible energy (Stapelberg et al., 2008). Reversely, if the demands on the minimum nutritional requirements in the forage do not meet with reality, it will be followed by weight loss, reduced animal's fertility, lowered lactation period, decreased reproductive rates as well as rising susceptibility to diseases and parasites due to weakened immune system (Olson et al, 2010).

As same as plant nutrients, animal essential nutrients are divided by required amounts on macro and micro-nutrients. However, the categories differ in some extent from plant nutrients and their classification is more unclear and depends on authors interpretation. For this reason, I will cite the categorization of animal nutrients by Whitehead (2000). Thus, the macronutrients for animals are: Calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), chlorine (Cl). The nitrogen is not mentioned here, because it is not ingested in mineral forms (as it is in plants), but is taken up in organic compounds as proteins, peptides, amino acids and other. The micronutrients are: iron (Fe), iodine (I), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), cobalt (Co), selenium (Se) and fluorine (F). However, there are a lot of other nutrients which are being considered to be essential in minute amounts, so called ultra-trace elements (or trace elements) but their importance is still not proved. They are for example: boron (B), silicon (Si), nickel (Ni), arsenic (As), chromium (Cr), cadmium (Cd) etc. (Soetan et al., 2010; Suttle, 2010). In conclusion, for the final recapitulation of both plant and animal nutrients it should be said that in either case the major body forming elements are: Carbon (C), hydrogen (H) and oxygen (O). The animal and plant macronutrients are the same, with exception of addition of sodium (Na) and chlorine (Cl) in animal macronutrients. The micronutrients Fe, Mn, Cu, Zn, Co and Mo are the same for both plants and animals. Boron is only plant micronutrient and contrariwise I, Se and F are only animal micronutrients.

2.4.1 Function of animal nutrients and their requirement in diet

The animal nutrients requirements and demands strongly vary during animal development (ontogenesis), physical (physiological) stress periods (e.g. mating season, pregnancy, parturition, lactation), as well as depend on the gender and animal species. Due to this, the accurate value of animal nutrients demands is hardly to determine. Nevertheless, there exist some techniques how can be quality of diet determined or at least well estimated. There are for example, invasive methods, such as oesophageal fistulae, stomach analysis or noninvasive methods represented by faecal analysis (see more in chapter 3.6). Especially, nitrogen and phosphorus faecal concentrations are good indicator of dietary proteins, especially for grazing animals (due to lesser amount of tannins in their diet) (Stapelberg et al., 2008). For example, the critical faecal nitrogen concentration for ruminants is between 11-12 g kg^{-1} dry matter, i.e. (1.1- 1.2%), in order to maintain rumen fermentation (Grant et al., 1995). More recently, it is suggested that faecal nitrogen concentration within the interval of 13- 16 g kg^{-1} dry matter is above the threshold of dietary deficiency avoiding nutritional stress (Wrench and Meissner, 1997; Grant et al. 2000). Critical faecal phosphorus concentration for most herbivore species is considered to be 2.0 g kg^{-1} (1.2%), albeit it was ascertained that if faecal P concentration value is between 1.9-2 g kg^{-1} for longer period of time the animal disorders as well as lower fertility appear (Grant et al. 2000).

Mechanism of mineral function is greatly complex starting from transport through cell membranes to multiplicity of functions, such as interference, synergism and substitution. Nevertheless, according to Suttle (2010) mineral functions in animal metabolism can be categorized on structural, physiological, catalytic and regulatory. The structural function mainly represented Ca, P, N, Mg, Si and S minerals, which form structural components and tissue in the body, such as bones and teeth (Ca, P, Si) and muscle proteins (N, P, S). The essentiality of nitrogen and phosphorus either in plant or animal organism was mentioned in chapter 3.1.1 as they constitute majority of essential biochemical molecules vital to metabolism function and reproduction, such as proteins, ATP, DNA, RNA and many others. Ca and P are minerals most abundant in bones and theirs required levels in the diet are 0.2-0.4% DM and 0.2% DM respectively. Optimal Ca:P ratio in food plant is from 1:1 to 2:1 for maintaining acid-base balance, nevertheless ruminants can tolerate even higher ratio if P requirements are met (Ohlson and Staaland, 2001). Minerals with physiological function are found in body fluids and tissue in form of electrolytes and they participate on osmotic regulation, acid-base balance, nerve impulse transmission and membrane permeability. They are for example: Na, K, Mg, Cl, Ca in the blood, but also cerebrospinal fluid and gastric juice have this function. Sodium is the main cation in extracellular fluids (e.g. blood plasma) of the animal body, whereas potassium (K) is the main cation in intracellular fluids. Na and K interact together, i.e. excess of K intake result in Na excretion. Na has important acid-base balance and osmotic regulation function. In stressed amount of Na, K, energy and protein, Pica behvaiour (perverted appetite-osteophagia, geophagia, carniovory) can occur (Underwood et al., 1940). Low concentration of P and Na in plant material can have negative impact on rumen microbial activity and thus reduced digestive efficiency. The dietary requirement of Na is 0.1 (DM) and for K 0.7 (DM). Risk of disorders become apparent at Na concentrations <90 mmol L^{-1} and K concentrations <38mmol L^{-1} in the saliva. Na deficiency can be also indicated by lower molar Na: K ratio in plants materials 0.07 as compared to 1.16 ratio in rumen and 0.17 in distal colon (Ohlson and Staaland, 2001). Catalytic minerals are able to catalyse enzymatic and endocrine reactions because they are part of metalloenzymes and hormones, or act as activator such as coenzymes, with both catabolic and anabolic activity as well as oxidant and antioxidant function. The minerals with catalytic functions forming metallo-enzymes/proteins and co-enzymes are represented by Fe, Cu, Mn, Se, Zn, Mo and Co. For example, Fe is crucial for oxygen transport by red blood, since is constituent of haemoglobin, as well as is important component of the cytochromes, which participate in cell respiration. Cobalt is essential component of vitamin B_{12} as well as enzymes cofactor involved in DNA biosynthesis (Soetan et al., 2010). Molybdenum effect in herbivore nutrition is more complex and with combination of high level of sulphur exerts limiting effect on Cu retention in animal organism, which can result in Cu deficiency syndromes, such as bone disorders. Finally, regulatory minerals can regulate cell replication as well as differentiation, e.g. signal transduction is influenced by calcium ions or selenocysteine influence gene transcription and thyroxine (essentiality of iodine) contribute to triiodothyronine transformation in thyroid gland, influencing physiological processes in the body such as growth, development, metabolism, heart rate and body temperature (Suttle, 2010).

2.4.2 Differences between digestive tract in ruminants and nonruminants

The ungulate herbivores are divided according to morphology of digestive tract on ruminants and non-ruminants (monogastric animals). Perissodactyla are strictly non-ruminant herbivores, with well-developed cecal digestive system (e.g. including *Equidae*) and the most species of ruminant herbivores is found in Cetartiodactyla order (*Ruminantia* suborder). These two digestive tracts have evolved in two strategies how plant material could be digested. The digestibility of plant materials is divided on easily digestible cellular contents, with almost 98% digestibility and hard digestible cellular walls comprise mainly of cellulose, hemicellulose and lignin. Cellulose is digested by rumen or cecal anaerobic microorganisms, while hemicellulose digestibility is diverse depending on their type and lignin is being considered as indigestible in anyway. The similarity of both digestive tracts consists in anaerobic fermentation of high fibre material by special anaerobic microbes, situated either in rumen or in cecum. However, the digestion of cellulose in ruminants is more effective than in non-ruminants, for example horses digestion is only 70% as efficient as compared to cattle and sheep (Hanley, 1982). The biggest advantage of rumen digestion resides in complex stomach (rumen, reticulum, psalterium, abomasum), providing the capability of regurgitation of cud and its repeated chewing resulting in smaller particle size suitable for better digestion, and pregastric fermentation. The anaerobic fermentation takes place in rumen, where symbiotic protozoa, bacteria and fungi transform the plant protein, starch and carbohydrates to higher quality animal protein as well as produce vitamin B complex and after that chymus enter to true stomach (abomasum) and intestines. Unlike ruminants, non- ruminants have postgastric fermentation situated in well-developed cecum. Hence, non-ruminants lose majority of microbial protein because the fermentation occurs after the main absorption site and thus only a small amount of microbial protein and vitamins can be recycled (Van Soest, 1982).

2.5 Foraging strategies of large herbivores

2.5.1 Diet selection and feeding types

Diet of large herbivores is functional link between habitat and feeding type strategies. The feeding strategies result from complex characteristic (parameters) such as herbivores body size and digestive tract morphology (ruminant vs. cecal), rumino-reticular volume to body weight (determines which food type is most efficient for processing) and mouth size (i.e. smaller mouth size is being related to browser and vice versa) as well as correspond to herbivores nutrient requirement and differences between nutritional content of fruits, browse and grass (Hanley, 1982; Danell (ed.), 2006). The feeding type strategies are categorized on frugivore (mainly herbivores in tropics), browser and grazer, which are broadened of mix-feeders, such as an intermediate frugivore-browsers and intermediate browser-grazers (Ganon and Chew, 2000). For example grazers are represented by ruminants such as catlle (*Bos taurus*), African buffalo (*Syncerus caffer*), Wildebeest (*Connochaetes taurinus*) and *Equiade* (horses, zebras, asses). Browsers are, for example, giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*) and mix-feeders can be represented by impala (*Aepyceros malapus*) and springbok (*Antidorcas marsupialis*) (Grant et al., 2000; Stapelberg et al., 2008). Diet decisions of large herbivores have to be compromise of spent time with foraging (time minimizing vs. time maximizing strategy, see Bergman et al., 2001) and the quality of forage, which means the proportion of required essential nutrients versus antinutrients (e.g. contents of secondary compounds and lignin) in food plants. Diet selection is often done against to antinutritive compounds in plant and/or plant parts, including food preference related to aversive post-ingested effect experience. This selection is often practise even despite of lower nutritional forage quality, which herbivores compensate by higher volume of ingested forage and/or by higher spectrum of grazing plant species (Provenza et al., 2003). Diet selection is also influenced by stoichiometry of particular elements in forage. Biological stoichiometry considers the concentrations of multiple chemical elements in living system and expresses them in particular element ratios (ratio of X to Y concentration) (Elser et al., 2010). Optimal stoichiometry Ca:P, S:N, Na:K, (Mo:Cu)*S in food plant for animal nutrition is very important for well-balanced diet and prevention of single mineral deficiency as well as negative impact of excessive intake of others mineral (Suttle, 2010). Neutral (NDF) and acid-detergent fibre (ADF), and acid-detergent lignin (ADL) are also important factors which are being considered in diet decision by herbivores. NDF represents cellulose, hemicellulose and lignin together, while ADF represents only cellulose and lignin, and ADL represents residual lignin.

2.5.2 Effects of seasonal nutrients fluctuation to large herbivores

Seasonal environment is typical for large part of the globe with rotation of favourable periods of plant growth and abundance and less favourable period with no plant growth, due to temperature variation or water limitation. Since, large herbivores select their habitat according to quality of their fodder, nutrients fluctuation in plants (see chapter 3.3.3) are underlying reason to seasonal migration, spatial distribution of large herbivores and additional foraging strategies (McNaughton., 1990; Frank et al., 1998; Stapelberg et al., 2008). Large herbivores adapt to seasonality by variety of life tactics including: timed breeding to calving in optimal condition for offspring survival, restriction of growth and development on period of food abundance, migration, food preferences and selection (young shoots, leaf, twigs etc.) or for example geophagia of the reason to obtain deficiency of some nutrients, which are presented in some occurrence in soil in higher amount than in plants (e.g., Na, Mo, Cu) (Freeland and Choquenot, 1990; Frank et al., 1998; Danell (ed.), 2006)

2.6 Methods how to acquire information from faeces

From the introductory opening, I would like to highlight the value of animal's faeces as an irreplaceable source of tremendous amount of information. Despite of fact that, the animal excrements are often considered as a waste products, they are the less demanding materials for the laboratory analysis and represent the most readable and available source of information. Faeces can provide information from ecological point of view, e.g. sex structure, age structure of population, population biomass, habitat use and range use, to dietary analysis and quality of diet of concrete animal species. Thus, these information is very useful from wide range of scientists, ecologist as well as to wildlife managers. In addition to this, even for the animals themselves, dung does not represent only a waste product. For example it may be used as an ownership marker or as a substrate for the chemical communication between individuals (Putman, 1984). So far, several techniques have been developed and used for dietary analysis from faeces, excluding the direct visual observations they are: microhistological technique, technique based on natural alkanes analysis of plant cuticular wax (e.g. Cuartas and Garcia-Gonzalez, 1996), NIRS- near infrared reflectance spectroscopy (e.g. Lyons and Stuth, 1992; Coates, 2000) and recently DNA faeces analysis (Pegard et al. 2009; Valentini et al, 2009; Pompanon et al., 2012). However, there are other methods which can be used from for faces analysis, such as the method of stable isotope composition of faeces, but it can only provide information about herbivores foraging strategy, i.e. whether they are grazer, browser or mix feeder (e.g. Botha and Stock, 2005; Codron et al., 2005). For determination of diet quality, the concentrations of macro and micro nutrients, NDF, ADF and lignin in faeces are frequently used as well (e.g. Wehausen, 1995; Wrench and Meissner,, 1997; Grant et al., 2000; Van de Wall et al., 2003; Stapelberg et al., 2008; Leslie et al., 2008; Verheyden et al., 2011). To determine animal's diet more precisely a number of other methods can be used co-operatively. For outline of what do these methods involve, two methods will be briefly described below as a representative examples of the older and modern methods of faeces analysis.

2.6.1 Microhistological technique for dietary composition

The microhistological examination is used for diet reconstruction and is based on observation and latter identification of different proportions of plant residues e.g. cuticle, found in faeces samples. The collected faecal samples are macerated and the dietary residues are observed and pinpointed under the microscope. Maceration is usually done in suspension of water and plant material is identified by using grid technique, also known as "point quadrat", and then found samples are identified by prepared set of reference slides. Such slides should be prepared before the main observing and it could be done by physical maceration of plant tissues in caustic chemicals e.g. sodium hydroxide or by using pepsin and cellulose solution to imitate digestion by herbivores. Overall, this method represents an essential outlook into the diet composition and animal's nutrition. Nevertheless, this method is demanding on time and labour and it can only provide an approximate estimation of diet composition, due to the fact that numerous fragments cannot be identifiable for their agglutination and opacity. Besides of mentioned problems with fragments identification, there is also problem with different digestibility of different feedstuff, which display in diverse representation in the faeces and thus the final results could be biased (Storr, 1960; Stewart, 1967; Putman, 1984).

2.6.2 DNA faeces analysis

DNA faeces analyses are recently developing methods characterized by high accuracy and precision of data determination for dietary studies. Up to nowadays, two DNA methods are developed: DNA barcoding by PCR uses and next generation sequencing (NGS) methods (Pompanon et al., 2012). The first mentioned method is based DNA extraction form faeces and selection of standardized DNA region (i.e. DNA barcode), which is amplified by PCR (polymerase chain reaction) method and after that are sequenced amplicon compared with prepared reference database for its identification. The NGS method is more recent and its biggest asset is the capability of direct characterization of numerous samples from sequences from PCR, which it can reveal simultaneously (i.e. DNA metabarcoding) (for more see e.g. Pegard et al., 2009; Valentini et al., 2009; Pompanon et al., 2012). So far, the DNA method for faeces analysis was rarely used due to financially demanding, but the ongoing development of NGS decreased its cost and widened the reference databases at the same time, which make promising prospect. The other contribution of DNA faeces analysis is that besides of providing information about consumed plant species, it can also provide information about animal gender from concrete faeces samples. The most essential and challenging step in DNA analysis is to choose appropriate barcode of plant material, for this purpose the most frequented is the uses of trnL (UAA) intron from the chloroplast, which is proved to be relevant for diet analyses of wild herbivores, because its universalism among plants and its high variability on the other side guarantee the consumed species identification (Valentini et al., 2009).

3 Aims

The aim of my thesis was to investigate functional link between content of macronutrients in soils and faeces of European bison (*Bison bonasus*) at three geographically distant localities. The initial hypothesis was that despite of the fact that plant and soil are in direct relation, meaning that accessible nutrients in soil are in direct proportion with plant nutrients, this relation between soil and faeces will not work directly, since herbivores can balance potential nutrient deficiency through foraging strategies such as diet selection and migration to another nutrients enriched location.

Particular aims were to determine:

1. **Functional relationship between concentrations of total N in soil and in faeces of European bison.**
2. **Functional relationship between concentrations of plant available P in soil and in faeces of European bison.**
3. **Mutual relationships of concentration of macronutrients (N, P, Ca, Mg, K) in faeces of European bison.**
4. **The difference in diet quality of European bison between summer and winter period in Cherga breeding station in Altai mountains (Russia)**

4 Methods

4.1 Localities and model animal

As model large herbivore species was chosen the European bison (*Bison bonasus*), where all sampled populations had to be minimally additionally fed. European bison is classified between grazer and intermediate feeder (see Figure 1). In summer season, European bison exhibits a degree of forage selection and grazes mainly forbs, sedges, and grasses (90% of diet). However, in winter season the woody species (trees, shrubs) increase in their diet (Borowski and Kossak, 1972; Hofman, 1989; Gębczyńska et al., 1991; Braukmann, 2011).

The own sampling was situated in the three various locations in Europe where European bison are found, i.e. in The Czech Republic samples were collected in the park Židlov, Russian samples were collected in Altai mountains in Cherga and last samples were collected in the Netherlands in Kraansvlak national park.

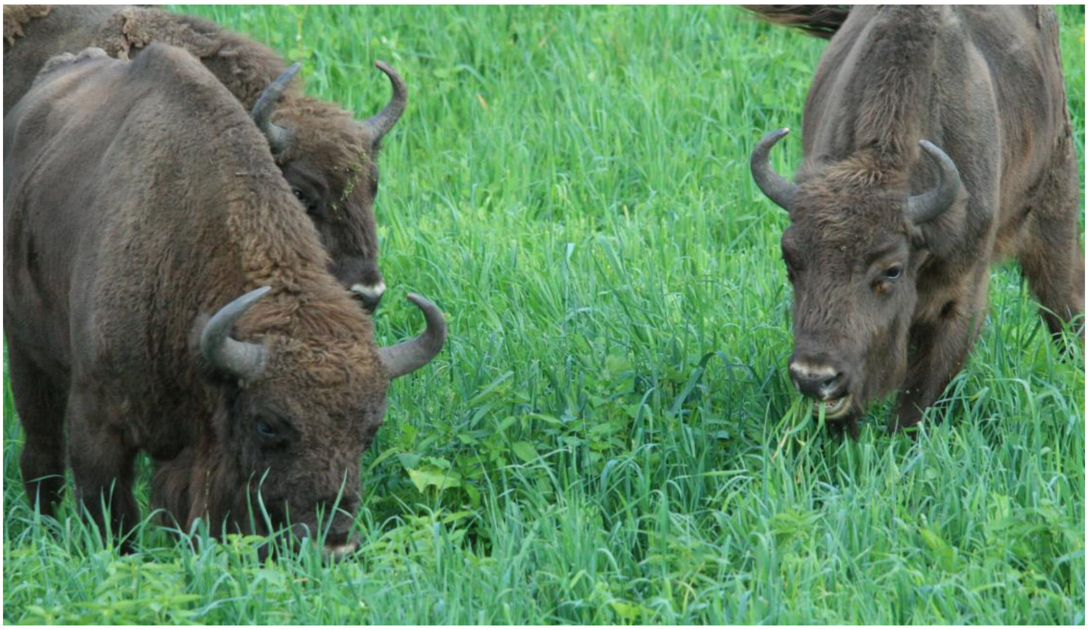

Figure 1 European bison foraging in Cherga breeding station. (Photo by Pavla Hejcmanová).

4.1.1 Židlov (Czech Republic)

(GPS 50.619444° N, 14.859167° E)

Židlov is fenced park on the former military area situated in Czech Republic, in the district of Česká Lípa near to city Mimoň (Figure 2). It is the second largest game park in Czech Republic with size of 3780 ha. The landscape is characteristic by sandy soil with predominance of forest (pine, spruce, beech, shrubs etc.) and open grasslands (Figure 3). Židlov is under the management of Vojenské lesy a statky České republiky (www.vls.cz.).

Figure 2 Satellite photo of the location of Židlov game park in Czech Rep. (Source: Google earth, 2014).

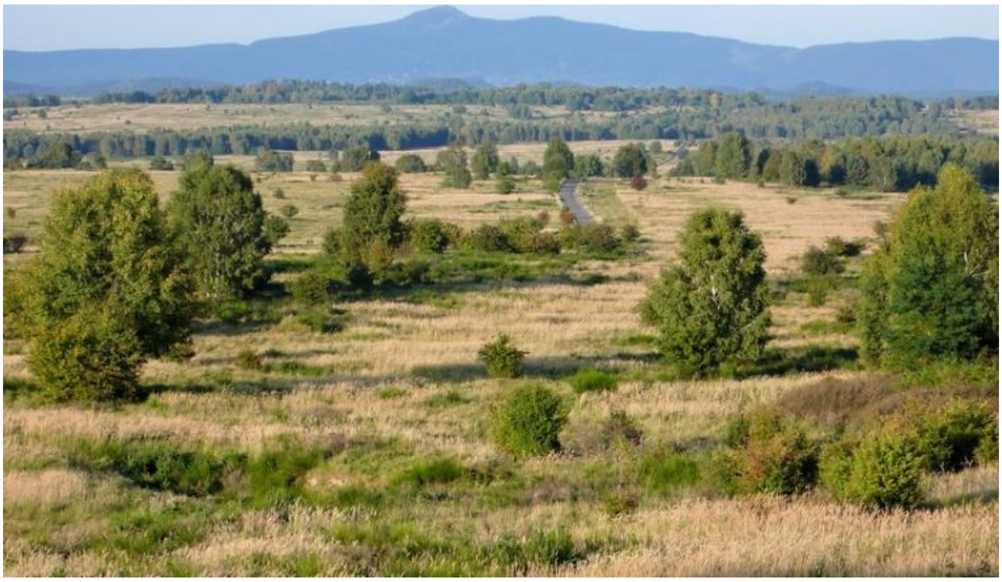

Figure 3 The landscape of Židlov game park in Czech Rep. (Source: www.vls.cz).

4.1.2 Kraansvlak (Netherlands)

(GPS 52°07'40.29" N, 5°31'43.32" E)

Kraansvlak is coastal dune area, situated in Netherlands, west of Amsterdam and is part of the Zuid-Kennemerland national park (Figure 4). It is the location of the European bison reintroduction pilot project, with approximately 15 bison (McCulla, 2012). It is a fenced area with size of 226 ha, with large variety of habitats from coastal dunes to old-growth forests (Figure 5, Annexes 6-8). The landscape used to be dominated by open sand and grasslands, however, in the last twenty years there has been a dramatic change in the landscape from limited shrubbery, open sand and grassland areas to an area that has lost almost all of the open sand areas, due to increased overgrowing (McCulla, 2012). Shortly before the bison were introduced, the area already consisted of 41% shrubs and trees, while only 57% were grassland and open sand. The remaining 2% were fresh water and marshland (Braukmann, 2011).

Figure 4 Satellite photo of the location of Kraansvlak as a part of the Zuid-Kennemerland national park in Netherlands. (Source Google earth, 2014).

Figure 5 The landscape of Kraansvlak national park. (Photo by Pavla Hejcmanová).

4.1.3 Cherga (Russia)

(GPS 51°33'56"N, 85°33'04"E)

Cherga breeding centre is situated in north- west in Altai mountains, with elevations 600–1400 m. above sea level (Figure 6). The average year temperature is +2–3°C (July +17°C, January –14°C) and vegetation period lasts 170 days. Annual precipitation is 500–700 mm (highest in summer) with snow moderate cover (Sipko, 2009). Cherga breeding station have three enclosures (50, 70 and 350 ha) with approximate 40 specimens of European bison (Worldwide Zoo Database, 2011) (see Figure 7 and Annexes 1-2).

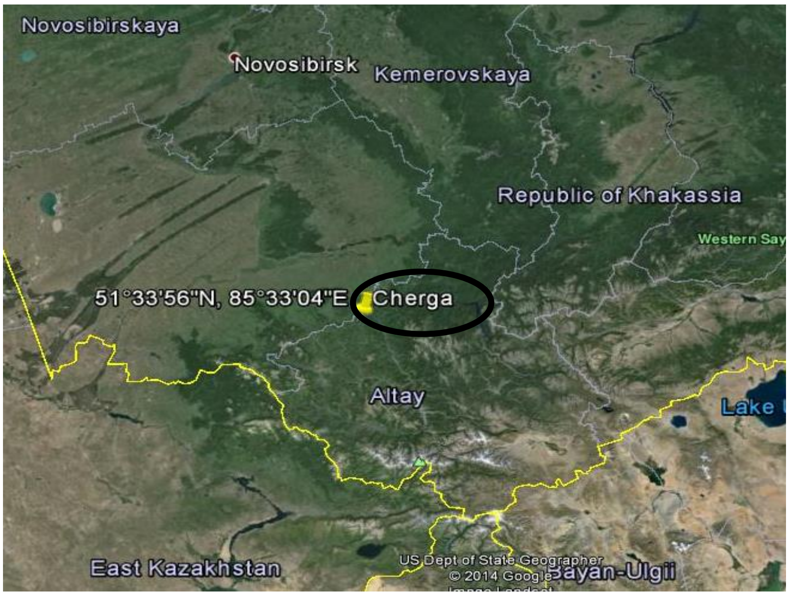

Figure 6 Satellite photo of the Cherga breeding station, Altai, Russia. (Source: Google earth, 2014).

Figure 7 The landscape of Cherga breeding station. (Photo by Pavla Hejcmanová).

4.2 Data collection

The procedure of sampling included collection of 40 faecal samples of European bison and 12 soil samples from three given localities (see Annexes 3-5). The collection of samples was done in Cherga in July 2011 and February 2012, Židlov in July 2013, Kraansvlak in August 2013.

4.2.1 Soil samples

Soil samples were collected in fenced areas where European bison were foraging. Samples had been taken from the upper 0 - 10 cm soil layer. From each Židlov and Kraansvlak one representative soil sample was collected. From Cherga region 10 soil samples were collected in summer. Subsequently, the soil samples were air-dried, grounded in a mortar, and sieved to 2 mm after removal of living roots. Samples were analysed in accredited Czech national laboratory Ekolab Žamberk (http://www.ekolab.zamberk.cz) for concentrations of plant available P, K, Mg, Ca in (mg kg^{-1}), total N (STN) and organic C (C_{org}) (g kg^{-1}). Determination of plant available concentrations of P, K, Mg, Ca was done by using Melich III extraction (Melich, 1984). The determination of total N was performed by using a TruSpec f. Leco instrument, where the soil samples were combusted at 950°. Organic C (C_{org}) concentrations were performed spectrophotometrically after oxidation in $K_2Cr_2O_7$ solution in H_2SO_4, at 135°. Soil pH (H_2O) was measured in suspension of 10g dry soil mixed with 50ml of distilled H_2O.

4.2.2 Faeces samples

From Židlov and Kraansvlak 5 representative faecal samples were collected, from each locality. From Cherga breeding station, 10 in summer and 19 in winter season samples were collected. Together 40 faecal samples, were collected. Faeces were collected specifically in fresh stadium and dung with beetles activity were excluded. Faeces samples were subsequently dried and sent to accredited national laboratory Ekolab Žamberk (http://www.ekolab.zamberk.cz) for analyses of concentrations of macro-elements (N, P, K, Ca, Mg), residual ash content (ash–P,K,Ca,Mg), neutral- (NDF) and acid-detergent fibre (ADF) and acid-detergent lignin (ADL) were determined as well. NDF represents cellulose, hemi-cellulose and lignin together, ADF represents cellulose and lignin. The N concentration was determined using an automated analyser TruSpec (LECO Corporation, USA) by combustion with oxygen in an oven at 950 ˚C. Combustion products were mixed with oxygen and the mixture passed through an infrared CO2 detector and through a circuit for aliquot ratio where carbon is measured as CO2. Gases in the aliquot circuit were transferred into helium as a carrying gas, conducted through hot copper and converted to N. Faeces samples were burnt in a microwave oven at temperature of 550 ˚C and weighed in order to determine ash content. After that were samples mineralized using aqua regia and P, K, Ca and Mg concentrations were then determined in the solution using ICP-OES (Varian VistaPro, Mulgrave, Vic., Australia). NDF, ADF and ADL contents were determined by standard methods of AOAC (1984).

4.3 Data analyses

The functional-link between concentrations of macronutrients N, P in European bison faces and total N and plant available P in soil (aims 1-2), were tested by simple linear regression in STATISTICA 12.0 program (StatSoft, Tulsa, USA). The soil (N, P) concentrations were used as independent variables and faecal (N, P) as dependent variables.

Unconstrained principal component analysis (PCA) in the CANOCO for Windows 4.5 program (Ter Braak and Šmilauer, 2002) was used to analyse mutual relationships among concentrations of N, P, K, Ca, Mg in faeces (aim 3). Data were log-transformed, centred and standardised in the course of the analysis. The results of the PCA analysis were visualized in the form of an ordination diagram constructed by the CanoDraw program (Ter Braak and Šmilauer, 2002).

The data for determination difference in diet quality between summer and winter period in Cherga (aim 4) were tested by Student’s t test for all macronutrients and fibre fractions separately in STATISTICA 12.0 program (StatSoft, Tulsa, USA). Unconstrained principal component analysis (PCA) in the CANOCO for Windows 4.5 program (Ter Braak and Šmilauer, 2002) was used to analyse of mutual relationships among concentrations of N, P, K, Ca, Mg in faeces of European bison between summer and winter period in Cherga. Data were log-transformed, centred and standardised in the course of the analysis. The results of the PCA analysis were visualized in the form of an ordination diagram constructed by the CanoDraw program (Ter Braak and Šmilauer, 2002).

5 Results

Soil and faeces samples were characterized by marked nutrients diversity due to element concentration levels which were very variable (see Tables 3, 4).

The soil concentrations of macronutrients, C_{org} and pH values (see Table 3) were highest in samples from Cherga, with exception of P, of which concentration was highest in Židlov (6.6 to 51 mg kg^{-1} respectively). Soil sample from Kraansvlak had lowest concentrations of N, P, K, Mg, in comparison to other two localities. The soils pH values ranged from 6.06 to 6.8.

The highest concentration from all faecal samples was for N and least for P and Mg. Similar to the Cherga soil samples, the peak of nutrients concentrations were in faeces from Cherga-summer period, contrary to Cherga winter period with minimum nutrient concentrations in faeces, for example max N concentration in summer 31.8 and min in winter 13.8 g kg^{-1}. In generally, faeces samples collected in summer had higher nutrient concentrations, in comparison to winter samples. The nutrient concentrations were similar in faeces form Kraansvalk and Židlov. However, the concentrations of NDF, and ADL were highest in Kraansvlak (see Table 4).

Table 3 Soil samples concentrations (means of total N, C_{org} and plant available P, K, Ca, Mg) and pH from studied loacalities (Russia- Cherga, Netherlands- Kraansvlak, Czech. Rep.- Židlov)

Soil	Russia, Cherga	Netherlands, Kraansvlak	Czech. Rep., Židlov
N (g kg^{-1})	4.86	1.09	1.65
P (mg kg^{-1})	6.60	1.00	51.00
K (mg kg^{-1})	253.00	83.00	111.00
Ca (mg kg^{-1})	5254.00	4178.00	1511.00
Mg (mg kg^{-1})	459.00	62.00	135.00
C_{org} (g kg^{-1})	207.00	5.80	1.37
pH	6.84	6.83	6.06

Table 4 European bison faecal concentrations (means ± standard error of mean, min and max) of N, P, K, Ca, Mg, NDF, ADF and ADL in (g kg^{-1}) at studied localities

Nutrients	Russia, Cherga-summer			Russia, Cherga-winter			Netherlands, Kraansvlak			Czech. Rep. Židlov		
	mean ± SE	min	max	mean ± SE	min	max	mean ± SE	min	max	mean ± SE	min	max
N	27.01± 0.92	21.56	31.83	16.30 ± 0.31	13.89	18.73	23.95 ± 0.72	21.77	26.14	22.41 ± 1.53	18.46	27.31
P	6.72 ± 0.39	5.40	9.00	2.78 ± 0.10	2.00	3.70	3.82 ± 0.49	2.80	5.20	4.60 ± 0.39	3.90	6.10
K	11.48 ± 0.54	8.60	14.40	4.68 ± 0.16	3.70	6.20	9.48 ± 0.92	7.10	12.20	5.08 ± 0.50	3.60	6.10
Ca	23.84 ± 0.62	19.50	26.60	13.18 ± 0.39	10.00	16.50	17.58 ± 0.87	14.70	20.00	19.84 ± 2.41	12.90	27.20
Mg	6.38 ± 0.23	4.90	7.30	2.39 ± 0.06	1.90	2.90	3.88 ± 0.13	3.50	4.20	4.52 ± 0.59	3.40	6.60
NDF	369.60 ± 20.47	299.00	489.00	605.68 ± 5.74	554.00	646.00	618.80 ± 18.23	573.00	663.00	514.80 ± 16.43	475.00	555.00
ADF	329.00 ± 14.42	269.00	416.00	490.05 ± 6.70	443.00	544.00	469.20 ± 9.17	446.00	493.00	406.80 ± 15.06	361.00	445.00
ADL	183.53 ± 4.45	157.50	206.60	132.44 ± 2.33	111.50	148.80	233.48 ± 2.73	227.20	242.90	214.34 ± 17.42	148.70	248.00

5.1 Functional relationship between concentrations of plant available N, P in soil and in faeces of European bison

There was significant relationship (P= 0.009) between concentrations of total N in soil and in faeces of European bison. Thus, the faecal N concentration increases with increased N concentration in soil (Fig 8-a). On the other hand, there was no significant relationship (P= 0.35) between concentrations of plant available P in soil and faeces (see Fig 8-b).

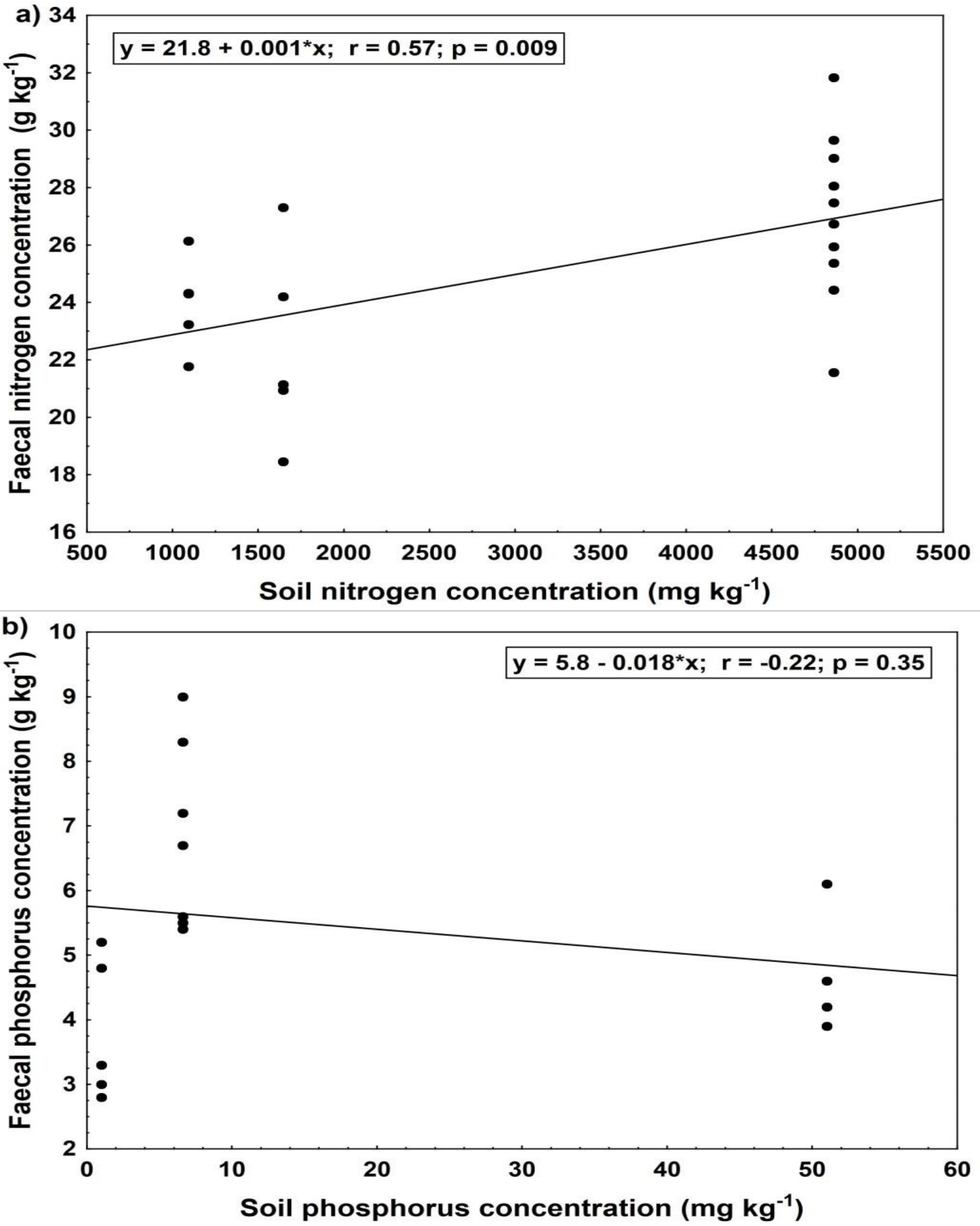

Figure 8 Simple linear regression of N, P, K, Ca, Mg of European bison faeces and concentrations of total N and plant available P, K, Ca, Mg in soil at studied localities (Russia Cherga- summer/winter, Netherlands- Kraansvlak, Czech. Rep.- Židlov)

5.2 Mutual relationships of concentrations of macronutrients (N, P, Ca, Mg, K) in faeces of European bison

The relationships in distribution of nutrients in individual faeces samples are shown in Figure 9. PCA analyses reveal that the first ordination axis explained 66%, the first two axis together 79% and four axis together 94% variability of faeces chemical composition. The faeces from Cherga (summer), are rich on macro elements (N, P, K, Ca, Mg) and have less content of NDF, ADF and ADL. In contrary to Cherga, faeces from Netherlands show higher relationship to concentrations of NDF, ADF and ADL and generally lesser amounts of macronutrients. The faecal samples from Czech Republic were not so uniform with trend to content more concentrations of NDF, ADF and ADL, but were most balanced on particular components concentration than faeces from other two localities.

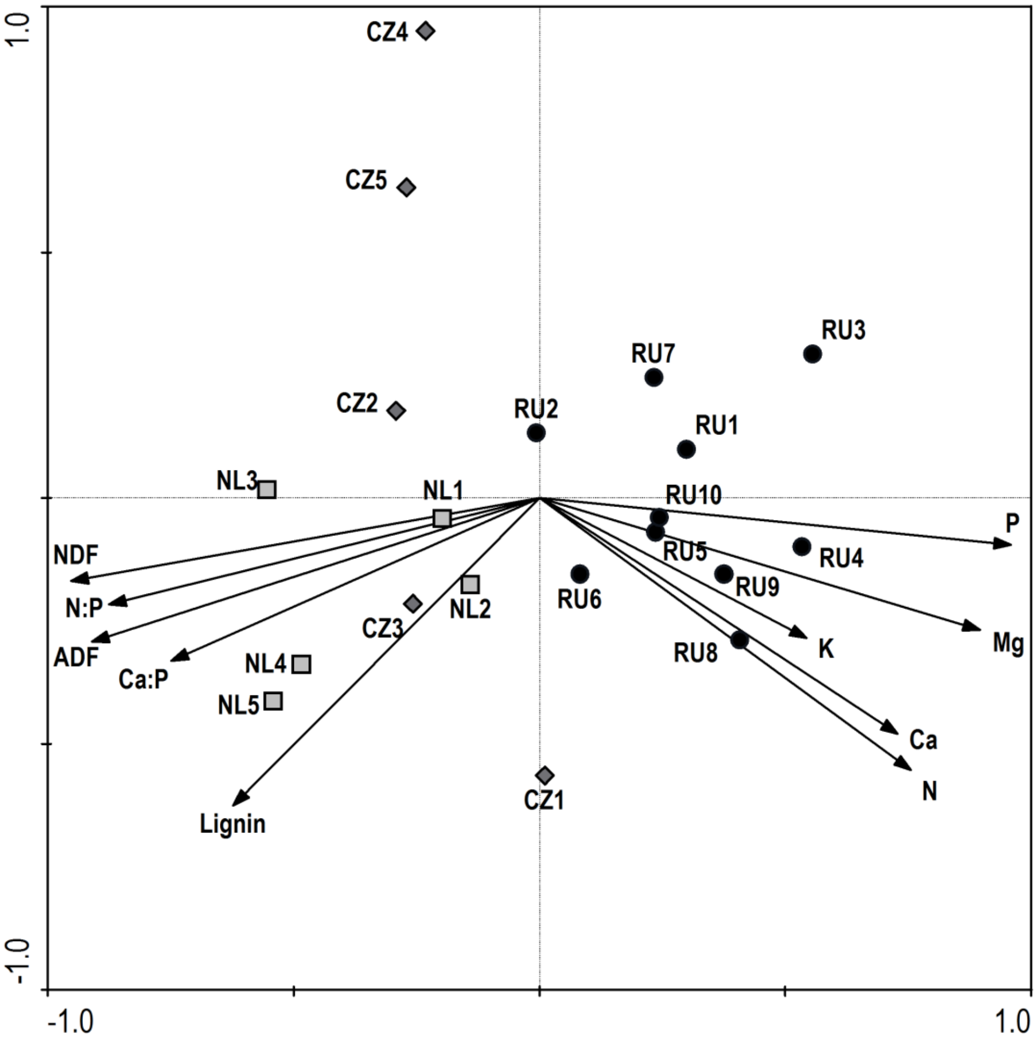

Figure 9 Multivariate analysis of mutual relationships of concentration of macro nutrients (N, P, Ca, Mg, K) in faeces of European bison.

5.3 The difference in diet quality of European bison between summer and winter period in Cherga breeding station in Altai

There were significant differences (all P< 0.001 Tab. 5) between nutrient concentrations in summer and winter faeces samples from Cherga. The total average nutrient concentration levels in faeces (N, P, K, Ca, Mg) were higher in summer period and declined to reach low values in the winter. Average concentrations of NDF and ADF markedly increased with the winter period (605 and 490 in winter to 369 and 329 g kg^{-1} in summer, respectively), although average concentration of ADL was slightly higher in summer (183 in summer to 139 g kg^{-1} in winter) (see Table 5). These differences between nutrient concentrations in summer and winter faeces samples from Cherga is shown in Figure 10 as well, where PCA analyses reveal that the first ordination axis explained 89%, the first two axis together 94% and four axis together 98% variability of faeces chemical composition.

Table 5 Student's t test of European bison faecal concentrations of N, P, K, Ca, Mg, NDF, ADF, ADL, N:P and ash in (g kg^{-1}) at the Russia, Cherga locality in winter and summer season

Nutrients	Russia Cherga, summer	Russia Cherga, winter		
	mean ± SE	mean ± SE	t- value	p
N	27.01± 0.92	16.30 ± 0.31	13.73	< 0.001
P	6.72 ± 0.39	2.78 ± 0.10	12.53	< 0.001
K	11.48 ± 0.54	4.68 ± 0.16	15.19	< 0.001
Ca	23.84 ± 0.62	13.18 ± 0.39	15.18	< 0.001
Mg	6.38 ± 0.23	2.39 ± 0.06	21.68	< 0.001
NDF	369.60 ± 20.47	605.68 ± 5.74	-14.18	< 0.001
ADF	329.00 ± 14.42	490.05 ± 6.70	-11.61	< 0.001
ADL	183.53 ± 4.45	132.44 ± 2.33	11.26	< 0.001
N:P	4.08	5.94	-8.64	< 0.001
Ash	306.5	115.47	19.04	< 0.001

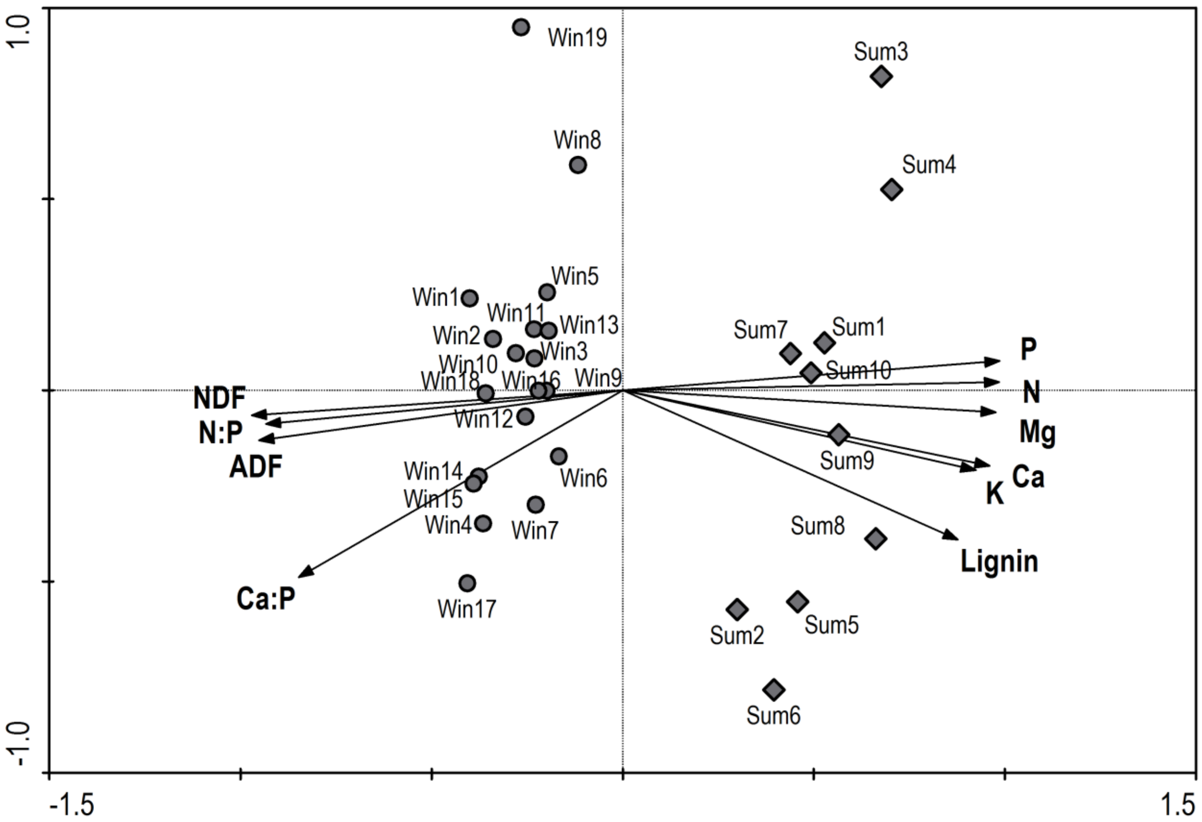

Figure 10 Multivariate analysis of European bison faecal concentrations of N, P, K, Ca, Mg, NDF, ADF, ADL (lignin), N:P and Ca:P in (g kg^{-1}) at the Russia, Cherga locality in winter (WIN) and summer (SUM) season.

6 Discussion

According to Wrench and Meissner (1997) and Grant et al. (2000), all sampled populations of European bison were high above the threshold of dietary deficiency. Critical concentrations for faecal nitrogen and phosphorus are considered to be 11-12 g kg^{-1} and 2.0 g kg^{-1} respectively, for most herbivore species (Grant et al., 1995; Wrench and Meissner, 1997; Grant et al., 2000). The average nitrogen concentrations from European bison faeces ranged from 16 to 27 g kg^{-1} and average phosphorus faecal concentrations ranged from 2.8 to 6.7 g kg^{-1}. However, there could be some misleading in faecal N, P concentrations due to presence of browse food species in European bison diet which content some amount of tannins. Thus, the accurate interpretation of faecal N, P concentrations as diet quality predictor decrease as browse species increase in the diet, due to capability of tannins binding proteins resulting in increased FN and FP (Hobbs, 1987; Wrench and Meissner, 1997; Grant et al., 2000). On the other hand, Leslie et al. (1987, 2008) were arguing that under natural feeding condition, browsers avoid to forage high on phenol substances and refer to a number of publications which indicate FN as a suitable proxy for nutritional status. There are no other studies focused on faecal N, P concentration as an index of diet quality in European bison, and thus our data cannot be compared.

The functional relationship between FN and soil total N was determined, however between FP and soil available P no relationship was found. This could be for several reasons: 1) because we did not have enough data, particular soil samples from Židlov and Kraansvalk localities 2) insufficient quantity of localities for analyses and 3) possibility of some bias due to effect of tannins (see earlier). However, despite of our initial hypothesis, there is some indication of possible relationships between soil and faeces of large herbivores as which was proved for nitrogen in this study. Therefore, more investigation is needed to be done in larger scale to confirm or disprove this finding.

From our research was ascertained, that localities play important role in content of macronutrients in faeces of European bison (aim 3). This prove faeces from Kraansvlak, which have highest concentrations of NDF and ADF, even compared to faeces from Cherga winter period, which refer to the dramatic changes in the Kraansvlak landscape due to increased overgrowing, mentioned in McCulla (2012). The faecal samples from Židlov were most balanced on content of all nutrients (Figure 9) and also the landscape of Židlov is represented by both open grasslands and forest area (see Figure 3 and Annexes 3-5). Finally, the faeces from Cherga were highest on the macronutrient concentrations, which correlate with the richness of vegetation, providing large spectrum of food including fertile grassland and seeded field by oat, as well as forest with trees such as birch, willow, bird cherry and etc. (see Figure 7 and Annexes 1-2). However, it could be surprising that faeces from Cherga summer period had higher concentrations of ADL in comparison to Cherga winter period, but this could be due to additionally feeding, which is practised during winter season and can lead to lower requirement of browse during winter than is without supplementary feeding (Kowalczyk et al., 2011).

7 Conclusion

The direct functional link between faecal nitrogen and total soil nitrogen was proved on faeces of European bison and soil samples from their habitats, thus reflecting similar pattern as plant-soil interactions. However, no functional link between faecal phosphorus and plant available phosphorus in soil was determined.

Furthermore, it was concluded (result 6.2) that localities play important role in content of macronutrients in faeces of European bison, and thus mutual relationships of macronutrients concentration (N, P, Ca, Mg, K) in faeces of European bison were strongly influenced by character of habitat and vegetation. Finally, the difference in diet quality of European bison between summer and winter period in Cherga breeding station was determined. The faeces from summer period have significantly higher macronutrients concentrations than faeces from winter period, which confirm the strong effect of seasonality on diet quality of large herbivores.

In conclusion, this research can bring the new insight in soil-plant-animal interactions through nutrients cycling in the Earth ecosystems, due to ascertained relationship between FN and soil total N. Such information could for example facilitate decision-making regarding management intervention and contribute to better comprehension to interconnection of nutrients flow. Therefore, more investigation in a larger scale would be appropriate to be done for confirm this result and test if more functional link between macronutrients from soil and faeces of large herbivores exist.

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Annexes

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