Department of Ecology
(Katedra ekologie)

THE DYNAMICS OF ELEMENTS IN SOIL AND PLANTS IN GRASSLANDS

(Ph.D. Dissertation Thesis)

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DEDICATION

To HIS Infinite intelligence: The Omniportent, Omniscience and Omniscience God,

&

My Family.

AUTHOR'S STATEMENT/DECLARATION

I declare that this PhD thesis of Department of Ecology has been completed independently, under the supervision of Prof. Dr. Vilém Pavlů. All the materials and resources are cited with regards to the scientific ethics, copyrights and laws protecting intellectual property. All provided and created digital data will not be publicly disposed without the consent of the Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague.

In Prague, March 2019.

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DEFINITION OF (OPERATIONAL) TERMS

This deals with further explanation about the meaning and prime focus of terminologies as used in this Dissertation Thesis.

Dynamics is used here to signify the prevailing variations, differences and/or changes in a given scenario.

Elements in soil and plants denote the macro/micro nutrients which in some cases includes the trace elements/heavy metals, and their general concentration trends in different soil and plants.

Grasslands represent areas dominated by grasses, legumes, herbs, etc and with less than 10% trees or shrubs.

Municipal solid wastes dumps represent the undesirable materials that are more of humanly generated than natural. They consist of day-to-day consumed and discarded items such as food wastes, containers, textiles, product packaging and other numerous wastes from residential, commercial, electronics, institutions and industries.

Land use is defined as the purpose to which the land cover is engaged mainly by man.

Mulching as used in this work could be defined as the process of covering the soil surface around the plants with various materials to create favorable conditions for the crop/plant growth.

Traditional cutting is a conventional method of trimming down the plant subtle height to a desired level to achieve the mandate of the grassland management.

C-S-R plants adaptation strategies or traits: These are classified ecological features prevalent in different plant species enabling their individual defined life survival in any given ecosystem.

Ellenberg nutrient indicator values (indicator values) is commonly used for rapid estimation of site conditions from species composition, especially when measured values of environmental variables are not available.

ABBREVIATIONS

HM = heavy metals

MSW = municipal solid waste

PTE = potential toxic elements

ICP–OES = inductively coupled plasma–optical emission spectrometry

ANOVA = Analysis of variance

FEPA = Federal Environmental Protection Agency

NURTW = National Road Transport Workers of Nigeria

RDA = Redundancy analysis

C-S-R plants adaptation strategies or traits: C = Competitors, S = Stress-tolerant, R = Ruderals

EIV = Ellenberg nutrient indicator values (indicator values)

HM = heavy metals

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SOC/OC/C_{org} = Soil organic carbon

STN/TN/N_{tot} = Soil total nitrogen

AL = arable land

FL = forest land

GL = grassland

SL = shrubland hills

UL = urban built-up green

WL = freshwater swamp and mangrove wetland

WB = Waterbodies

SRTM = Shuttle Radar Topography Mission.

DEM = Digital Elevation Model

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CHAPTER ONE

General introduction

1. General introduction

Grasslands cover nearly 37% of the entire earth's terrestrial surface (O'mara 2012), about 50% of African land area (Osborne et al. 2018), and approximately 30% of European agricultural land area (Huyghe et al. 2014) with large ecological and socio-economic benefits in terms of food and ecosystem services (Egoh et al. 2011; Dengler et al. 2014; Baudena et al. 2015). The grassland biome exits in temperate climate (temperate grassland with mainly the C3 plant species) and in the tropical climate (tropical savanna with mainly the C4 plant species). Apart from the biogeochemical factors, management has widened the gap between temperate and tropical grasslands in their productivity and sustainability with the later having poorer management. Several studies have reported about the changes in species richness and diversity which are caused by management practices in the tropical grasslands (Egoh et al. 2011; Shi et al. 2013; Barros et al. 2015) and temperate grasslands (Pavlů et al. 2011; Hejcman et al. 2010; Hejcmanová et al. 2016). Different methods of grassland management especially cutting, mulching, grazing, and unmanaged are known to influence plant species composition and species diversity differently (Pavlu et al. 2011; Gaisler et al. 2013; Gaisler et al. 2019), yet little is known about the prevailing dynamics in soil and plants chemical properties in these different grassland management. For instance, studies have reported that cutting with biomass removal can cause nutrient depletion from the soil, especially when performed over a long period without fertilization (Schnitzler and Muller 1998; Perring et al. 2009). Though the nutrient contents removed annually by biomass harvesting might be low (Bakker 1989), but given several years of continous harvest, the quantities of nutrients removed might be devastating. In comparison with the managed grasslands, unmanaged grasslands have soil nutrient availability that may be elevated by either the input of nutrients from atmospheric deposition, mineralization of soil organic matter or by weathering of soil minerals (Köhler et al. 2001). Seldomly, it is confusing to accurately predict the likely effect of management on soils. For example, it might be expected that management with mulching would support more nutrient turnover and herbage due to swift decomposition of the mulched biomass when compared with cutting or abandonment (Oomes et al. 1996), but the result could differ especially in long-term management experiment (Mašková et al. 2009). This is because, the biomass left on the sward might instead lead to further changes in the physical properties of the soil (Facelli and Pickett 1991) and conconsequently to unexpected changes in soil chemical concentrations. Therefore, it could be concluded that management regime as well as soil chemical properties are major factors that influence vegetation in temperate grasslands (Bakker 1989).

Grazing by livestock has been one of the common management methods for grasslands worldwide. Contrary to mulch and/or cut grasslands, grazed grasslands are affected by many factors namely trampling, seed dispersion, in-situ nutrient supplement by excretion, and selective defoliation by animals (WallisDeVries 1998; Rook et al. 2004; Ludvíková et al. 2014). Grazing often promotes spatial heterogeneity of the plant canopy, and the specific influence of grazing depend on the type of grazing animal, grazing intensity, as well as the stocking time and duration (Ausden 2007). Livestock could graze selectively on some species and on certain plant segments (Rook and Tallowin 2003), they create a spatial heterogeneous sward structure with a mosaic of various heights (Bakker et al. 1984). Typically, sward height is identified as a crucial predictor of plant responses to defoliation intensity (Diaz et al. 2001; Naujeck et al. 2005). The species composition and heterogeneity of flora in temperate grasslands are often linked with grazing pressure and animals' preferences (Pettit et al. 1995) and subsequently a structure with patchy swards (Ludvíková et al. 2014).

However, there are numerous grassland management practices such as mulching, fertilization, regulated cutting, and sustainable grazing in the Central Europe and other developed regions, but in Nigeria and other sub-Sahara African (SSA) regions improper defoliations especially indiscriminate grazing, cutting, burning and litter removal are common. The savanna soil is not only becoming poor in vital nutrients but are also depreciating in ecosystem services including nutrient turnover with decreasing plants and microbial activities (Sanchez 2002; Vanlauwe et al. 2002); a situation which is entirely different from the temperate grasslands' scenario (Hejcman et al. 2013). Many factors including dearth of information, illiteracy, poverty, and negligence by government and agricultural institutes are the likely reasons in SSA. For example, long-term unregulated dumping of municipal solid wastes on grasslands in Nigeria is one of the pathetic proofs of illiteracy, poverty and absolute negligence by the stakeholders in agriculture and nature conservation.

Municipal solid waste dumps and dynamics of elements in the soil and plants

On grasslands, municipal solid wastes (MSW) create more harm than good to the ecosystem. MSW are deregated materials that are primarily of anthropogenic than natural origin. The wastes are generated from the daily consumption and disposal of substances such as food wastes, containers, textiles, product packaging and other forms of wastes from residential, commercial, electronics, institutions, and industries (Singh et al. 2011; Amos-Tautau et al. 2014; Oketola and Akpotu 2015). The persistent build-up of the MSW is linked with heavy metals (HM) formation in the soil and plants. Though, metals in required amount are required for survival by plants and animals yet, their high concentrations in the soil lead to toxicity of the ecosystem (Alloway 2004; Kabata-Pendias

2010; Ali et al. 2013; Ogbonna et al.2013; Liang et al. 2016). HM from the MSW can have negative effects on the soil microbes and fauna, and consequently limiting the contents of the essential nutrients' availability for plants' uptake (Behera et al. 2013; Ogbonna et al. 2013; Oketola and Akpotu 2015). Therefore, to restore the grasslands productivity in this case, empirical research work is necessary while, soil remediation processes are critically needed.

Effects of mulching, cutting and grazing on soil and plants in grasslands

Mulching could be defined as the method of cutting the aboveground sward without herbage removal and crushing the plant residues into smaller particules which are subsequently applied uniformly onto the soil/grassland surfaces by spreading (Doleˇzal et al. 2011; Metsoja et al. 2012). In return, the plant biomass over time decomposes and releases nutrients into the ecosystem for soil enrichment and plants uptake (Gaisler et al. 2004; Pavlů et al. 2016). The importance of mulching has been reiterated by many authors who described the method as a good way of enhancing soil fertility by increasing moisture content with decrease soil surface evaporation, soil temperature attenuation, and weed reduction (Sainju et al. 2005; Murungu et al. 2011; Prosdocimi et al. 2016; Thankamqini 2016). In Central Europe especially Czech Republic, mulching has been widely adopted by the farmers since 1990s as a low-cost alternative (Dux et al. 2009) to either grazing or conventional mowing. This practices since then has been either recommended to prevent unwanted changes in botanical composition of grasslands that are not presently used for agricultural purposes (Fiala 2007; Gaisler et al. 2013), or as an alternative management for the conservation of species-rich grasslands (Kahmen et al. 2002; Moog et al. 2002). However, many studies have compared mulching conducted once or twice per year, with cut and unmanaged treatments (Bakker et al. 2002; Kahmen et al. 2002; Duffková 2008; Maˇsková et al. 2009), but only few studies have been performed on long-term period in the highlands of Czech Republic (Gaisler et al. 2013; Pavlů et al. 2016; Gaisler et al. 2019).

Contrary to the common practice in Czech Reublic and in most European countries, plant residue mulching is rarely practiced in SSA instead the straw-mulching. Straw-mulching has been reported for slow decomposition, promotion of termites' attack on plants, and poor nutrient supply (Wood et al. 1980; Mando et al. 1999; Mando et al. 2001; Nyagumbo et al. 2015).

Although, both plant residues mulching and cut with biomass are sustainable grassland management methods used for promoting soil quality and species diversity (Moog et al. 2002; Gaisler et al. 2004; Prosdocimi et al. 2016), yet strw-mulching and cut with biomass removal predominate in SSA especially Nigeria (Manyong et al. 2001; Babatunde et al. 2012). This is attributed to increasing poor population leading to high rate of plant residues usage for domestic purposes such as for building and fuel, and this without fertilization could consequently cause a decline in soil fertility and plants richness (Buerkert et al. 2000; Chikoye et al. 2000; Manyong et al. 2001; Savadogo et al. 2007; Babatunde et al. 2012; Dossou-Yovo et al. 2016). A recent study by Gaisler et al. (2019) reported that traditional management of two-cuts with biomass removal was the best alternative for conserving plant species richness and diversity in Czech Republic. In contrast, the result is different in most countries in SSA because the biomass is removed by burning with intense heat penetrating the deep soil profile subsequently reducing microbial activities (Sainju et al. 2005; Murungu et al. 2011; Prosdocimi et al. 2016; Thankamqini 2016).

Grazing is considered as one of the best means to improve soil quality (Virgona et al. 2006; Teague et al. 2011; Chen et al. 2015; Xu et al. 2018; Zegler et al. 2018) and promote plant diversity by suppressing competitively dominant plant species and fostering rare species (Crawley 1997). Several studies in Czech Republic (Pavlů et al. 2006; Pavlů et al. 2009; Golodets et al. 2013;

Ludvíková et al. 2014; Ludvíková et al. 2015; Hejcmanová et al. 2016), and in Nigeria (Lal 1996; Buerkert 1998; Asadu 1999; Harris 1999; Ayuba 2001; Aremu 2007; Oladele and Braimoh 2014; Jimoh et al. 2017; Lawal-Adebowale et al. 2018; Nwaogu et al. 2019) have been conducted on the strong intractions between grazing, nutrients in soil and plants, and species composition. Though grazing had positive effects on the soil nutrients and plants species richness, yet this to a large extend depends on the grazing intensity. For example, light to moderate grazing could enhance soil C through increased plant productivity by reinstating aging or dead plant tissues with active photosynthetic tissues (Holland et al. 1992; Zhang et al. 2015b) and via prolonged light exposure on younger plant tissues, extending C capture during daylight periods (Shao et al. 2013). On the other hand, heavy or intensive grazing may lead to excessive hoof trampling by grazing animals causing soil compaction, which could result in decreased soil pore space, diminished infiltration, and low plant available water (Willatt and Pullar 1984; Tate et al. 2004; Kotzé et al. 2013; Pulido et al. 2016). These impacts might in turn surpress root and mycorrhizal growth (Barto et al. 2010), mar soil structure (Steffens et al. 2008), disrupt C and N cycles (Piñeiro et al. 2010), and consequently decrease productivity of the grassland (Byrnes et al. 2018).

Apart from grazing intensity, type of livestock has been identified as another factor which has crucial influence on soil properties and vegetation (Rook et al. 2004; Wrage et al. 2011). Livestock such as cattle and sheep differ in terms of their grazing characteristics based on their nutritive requirements, body size and muzzle structure. Cattles are known to graze patchily, whereas sheep could select single plants and plant parts (Illius & Gordon 1987; Rook et al. 2004). On the other hand, chickens have been identified as good pasture livestock with vital influence on soil properties and floristic compositions (McCall 1980; Grichar et al. 2005; Su et al. 2018). Unlike ruminant herbivores, chickens are omnivores that can derive various foods (such as insects, fresh leaves, invertebrates, buds, grains and stones) from grasslands (Lomu et al. 2004), and consequently redefining soil and plants characterizations (Su et al. 2018). Their inherent scratch-feeding mode promotes soil surface aeration and the incorporation of organic matter into the soil (Fukumoto 2009). Poultry manure has multiple values in agriculture. For example, as a sustainable means of organic fertilizer to the soil (Sivakumar 2006; Su et al. 2018), and as source of supplemental N, fibre and energy to ruminants (Mishra et al. 2015). Some authors have also reported the use of poultry litter as an alternative for natural fuel source. The authors documented that poultry litter with less than 9% water contents can burn without extra fuel which can be suitably used for energy generation (Jayathilakan et al. 2012). Notably, free-ranging chicken production could increase labile manure to degraded soil, thereby enhancing plant growth in the poultry farmlands (Grichar et al. 2005). In Jiangsu, China for example, Li et al. (2011) found that the soil treatment with poultry litter significantly increased the soil macropore (mac-P), mesopore (mes-P) volume and SOC when compared with the chemical fertilizer treatment. The authors further reported greater soil total porosity, improved soil pore structure, and enhanced soil microbial activities in the soil applied with poultry litter relative to the inorganic manure site.

Excreta from chicken grazing (CG) amiliorates many soil-associated problems with its use as manure by lowering moisture content, reducing odor, improving texture, subduing weed-seed viability, and providing uniform and stable particles that are easier to handle (Schelegel 1992; Dao 1999). Other studies added that poultry manure has been essential in improving the fertility of the cultivated soil by increasing the organic matter content, water holding capacity, oxygen diffusion rate and the aggregate stability of the soils (Mahimairaja et al. 1995; Adeli et al. 2009).

Indeed, nutrients provided by poultry litter have been reported by many authors to have positive effects on soil and plants growth (Mitchell and Tu 2005; Reddy et al. 2007). However, optimum use of this manure requires knowledge of the composition not only in relation to beneficial values but also to environmental implications. This is because, continuous droppings of poultry excreta or an intensive introduction of the manure might intensify the concentrations of potassium phosphate (K_3PO_4) and nitrate (NO_3^-), and consequently leading to leaching and eutrophication, nonpoint water contamination, and groundwater pollution (Zhu et al. 2004; Adeli et al. 2007; Kobierski et al. 2017).

Generally, livestock grazing (whether cattle, sheep or chicken) is associated with contrasting soil properties (Rook et al. 2004; Wrage et al. 2011), varying vegetation composition (Nwaogu et al. 2019), and spatial heterogenous sward structures (Pettit et al. 1995; Ludvíková et al. 2014). Therefore, an understanding of the interactive effects of grazing types/forms on soil and plants nutrient status as well as species composition is pertinent for sustainable management of the grasslands.

In these contexts, this PhD Thesis is focused on ***appraising the dynamics of elements in the soil and plants in grasslands under different management (such as gazing, cutting, mulching, and waste disposal)***. The conception of this work was prompted by the need to conserve the grasslands and their indispensably associated ecosystem services. As the human population increases, extra food is needed from the world's existing agricultural land base (especially grasslands). Grazers are efficient converters of forages and poor-quality feeds into humanly edible energy and protein, and grassland-based food production can produce food with a comparable carbon footprint as mixed systems (O'Mara 2012). Grasslands are not only sources of food but are very important carbon sinks, groundwater purification, and socio-cultural services. It is therefore expedient to investigate the nutrient contents and their disparities in plants and soil under unmanaged waste sites, in mulching and cutting regimes, and under different grazing systems. This will help to provide the agriculrurists with vitally substantial information necessary for proper management, maintenance and restoration of degraded grasslands.

Conclusion

This chapter on general introduction is concluded by emphasizing that the thesis is structured into eight chapters and each chapter ends with list of citations except the last chapter. Chapter one dealt with the general introduction which briefly discussed the main concept of the work by relating to each of the case studies involved. The chapter further highlighted the thesis main goal, objectives, research questions and hypotheses (where applicable). Other components of the chapter one includes the brief overview of the limitation of the work, methothodology, study areas, data, and results. Chapter two to chapter six addressed the five case studies performed in accordance with the main goal of the Thesis. Meanwhile, these case studies have been either submitted to/or published in reputable ISI journals indexed in web of science (WoS) database with scientific impact factors. Chapter two focused on the '*variations of elements in soil and plants due to municipal solid waste dumps in grasslands, Nigeria*'. The unawareness of the values of the grasslands by the people and the government was shown by the act of indiscriminant dumping of wastes without considering the effects on the soil and plant species. As earlier mentioned, the case study in chapter two was performed to correct the negligence on the part of the people and government by using the information from the result as evidence. The research is a good beginning to reveal the need to conserve and restore the grasslands by preventing their contamination with municipal wastes in the region. Chapter three examined the '*differences in SOC and STN status in grassland and other neighboring land use, Nigeria*'. This study did not only help in the comprehension of the dynamics in concentrations and stocks of SOC and STN in the grasslands, but it also enabled a comparative analysis between the grasslands and other land use types in the study area. The knowledge from this study will support in the proper management of the grasslands and other neighboring agricultural lands in the watershed. Chapter four evaluated the '*responses of plant species composition to long-term effects of mulching, traditional cutting and no management of improved upland grassland, Czech Republic*'. This research has the longest time frame among all the studies performed during this PhD programme. It covered 13 years of investigation on how the various meadow management approaches influence long-term successional changes in plant species composition, and species richness in a previously improved upland meadow in Jizera Mts. Chapter five investigated the '*effects of grazing and dung on nutrient level in herbage and soil'.* Performed at Jizera Mts in Liberec, this study examined whether the presence of faeces, deposited by heifers, on tall sward-height patches would significantly affect the amount of nutrients in the soil and consequently in the herbage of *A. capillaris* grassland. Chapter six assessed the '*soil and plants status in a chicken grazed grassland'* in Netluky village, Czech Republic. The study attempted to find out the impacts of the poultry livestock on the grassland, and to ascertain if there is any contrast or similarity in the soil chemical properties and botanical composition before and after grazing. The result could help to consolidate the introduction of chicken as a grazing livestock for sustainable grassland management.

1.1 Main goals, research hypotheses and questions

This main goal of this study is to appraise the dynamics of elements in the soil and plants in grasslands under different management (such as gazing, cutting, mulching, and waste disposal). Therefore, the variations in the concentrations of elements in the soil and plants in grasslands are treated in two approaches, namely;

-Management approach (response of nutrients in the soil and plants to grazing, cutting, and mulching);

-Pollution approach (reaction of elements in the soil and plants to anthropogenically generated wastes).

Investigating the dynamics of macro and micro nutrients in the soil and plants under the two approaches enabled the postulation of some hypotheses and questions. These research hypotheses and questions were framed based on individual case study and structured to achieve the main goal of the thesis.

In ***case study one***, it was hypothesized that in addition to the significant effects on the soil and plant chemical properties, the MSW dumps affect the species composition and richness.

In ***case study two***, it was hypothesized that SOC and STN concentrations and stocks are significantly affected by slope position, and soil bulk density and depth.

Case study three was suppositioned with the question: how do different meadow management methods (mulching, cutting and abandonment) affect long-term successional changes in plant species composition, the main plant functional groups, and species richness?

Case study four had the hypothesis that: the presence of faeces, deposited by heifers, on tall sward-height patches would significantly affect the amount of nutrients in the soil and consequently in the herbage of *A. capillaris* grassland.

Case study five hypothesized that chicken grazing (CG) caused increased soil N while, preferential grazing by the chickens led to changes in botanical composition.

1.2 Study areas

Briefly the study areas will be given an overview here.

The studies were performed in two different ecological regions: (1) temperate and (2) tropical climates.

(1) *temperate region (Czech Republic)*

The studies in the Czech Republic were performed in two different areas; in the village of Oldřichov v Hájích, and in the Netluky village. Case studies three and four are located in the Jizerské hory Mountains (Jizera Mountains) with 420 m altitude a.s.l., in the northern Czech Republic, which is 10 km north of Liberec (50°50.34′ N, 15°05.36′ E) in the village of Oldřichov in Hájích. The experimental site was established in 1998 and has a mean annual temperature of 7.2 °C and an average annual precipitation of 803 mm (Liberec Meteorological Station). The site has a medium deep brown soil (Cambisol) with 10-15 cm and is underlain by granite bedrock. The case study six (chicken grazing experiment) was conducted on 0.7 ha of experimental grassland at Netluky village (150°2'21.344"N, 14°36'51.075"E). The altitude of the study site was 284 m a.s.l., the average annual precipitation was 591 mm and the mean annual temperature was 8°C with loamy clay soil texture.

(2) *tropical region (Nigeria)*

The case studies one and two experimental sites are located in Nigeria. The case study one (soil and plants responses to MSW) cut across Nigeria (4^0N -14^0N, 3^0E -15^0E). It covered selected grasslands along the major roads linking the sub-urbans and the cities. The area has an annual temperature ranging from 23 ^{0}C to 40 ^{0}C (from the South to Northern part), and annual rainfall ranging from 500 mm -3000 mm (from the North to the South). The soils are of loamy-sand properties and primarily of Luvisols, Vertisols, Lithosols, and Ferralsols taxonomical groups (FAO, 2001). The dump sites were between 15-20 years old, and 3-8 hectares in area. The dumpsites are located in relatively plain land with about 60-90 meters above sea level. The case study two (on the differences in SOC and STN stocks) is found in Imo watershed located in the south-east and south-south regions of Nigeria with geographical extends between 4° 50' 00''N to 6° 02' 00''N latitude and 6° 04'10''E to 7°34' 15''E longitude. It has an area of 4321.4 km^2, and an elevation ranging from about 52 m - 340 m a.s.l. The watershed is in a humid tropical climate having a mean annual temperature of 28.5° C, and a mean annual rainfall of 2400 mm with over 70% falling between the months of May and October (FORMECU, 1998). It has a sand, loamy-sand textural characterization (Udom and Ogunwole, 2015).

1.3 Materials and Methods

The differences in the study locations prompted the use of several materials and methods to achieve the main goal of this work. Rising plate meter was used to measure the plants height; Cutlasses, hoes, and rakes were used to clear vegetative cover from the experimental sites before soil sampling. Terrax and terra-scoop in combination of a Dutch soil auger and a spatula was used to collect soil samples from the dump sites; Inductively coupled plasma–optical emission spectrometry-ICP–OES (720 Series, Agilent Technologies) was employed in the laboratory by the help of the lab-attendants for determining the plant available nutrients after extraction by Mehlich III (Mehlich, 1984). ArcGIS 10.1, NigSat and USGS Landsat images were for land use types identification/classification. Hand-held GPS (Garmin GPSMAP 64ST, USA) was used to locate/identify sampling points. Shuttle Radar Topography Mission (SRTM) for DEM at 30 m resolution was used for slope/altitude validation process. A tractor-driven mulching machine (Uni Maher UM 19, Gerhard Dücker GmbH & Co. KG) was used for the mulching treatments. Plant biomass was crushed into pieces 5–10 cm long, spread on the sward surface and pressed by the roller. Others are softwares such as the statistical packages namely; STATISTICA 13.1 (Dell Inc 2016), CANOCO 5 program (ter Braak & Šmilauer, 2012), and IBM SPSS 20.0 (IBM, 2011) which were used for the statistical analysis. These have programs such as ANOVA and RDA which were applied to determine the mean differences and significance level between treatments and sites in time. They were also used in testing the relationships such as regressions and correlations among measured variables. In terms of the experimental design, because of the diversity in the ecological settings of the work, diverse methods were applied as appropriate to each case study in order to achieve the main objective(s). This information is shown in detail in the case studies (shown in chapters two to chapter six).

1.4 limitation of the work

In respect to the vegetation data, the samplings focused only on the aboveground plant residues. However, not including the below ground herbage biomass does not mean that it has no influence on the above ground plants structure, but due to the schope of the study.

1.5 Results and conclusions

The results from the papers and manuscripts presented were concrete evidence that the concentrations of the elements (whether essential or trace element) differed in soil and plants in grasslands. The findings further revealed that the soil properties and species compositions varied due to the differences in human activities such as contrasting management regimes (grazing, cutting, mulching) and contaminants from anthropogenically dumped wastes. On this note, from the five case studies the following conclusions were drawn that:

- the MSW significantly increased HM concentrations which consequently affected the chemical properties of soil and plant as well as the species composition and richness;
- SOC and STN concentrations and stocks were significantly affected by slope position, soil bulk density and depth;

- repeated mulching cannot completely be an alternative for traditional two-cut management in improved upland meadows without decreasing plant species richness and diversity, as well as changing the sward structure;
- the presence of faeces increased the P and K concentrations in the herbage in tall sward-height patches in intensively grazed pasture only, whereas no effect of faeces was found in extensively grazed pasture., which is likely due to a "dilution effect".
- though, chicken grazing caused increase in soil N yet, their preference in forage grazing induced changes in botanical composition.

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CHAPTER TWO

Assessment of the impacts of municipal solid waste dumps on soils and plants

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Abstract

The study aimed at evaluating the impacts of open municipal solid wastes dumps on soil and vegetation near the main roads linking major cities in Nigeria. We hypothesized that the metals from the wastes exerted substantial impacts at the dump sites which affect the soil and plants. Data was analyzed from five dump sites and five control sites. The result revealed that the effects of the heavy metals (HM) were significant and higher at the dump sites where their concentrations were far above the EU, and Canadian environmental quality permissible limits for agricultural soils and vegetation. In contrast with dump sites, a significant relationship ($R^2 = 0.70$; $p < 0.001$) was found between the number of plant species and area at control sites. Shrubs and herbs were more tolerance with higher contents of HM compared with grasses. Plants leaves showed more HM contents compared to the shoots or roots. The soil and plants contents of the HM were relatively in the order of Zn > Cr > Pb at both dump sites and control sites. Further study on the effects of more HM on soil and plant is recommended in the area. Recycling and bio-phytoremediation processes should also be introduced.

Key words: Soil, plants, soil contamination; potential toxic elements; open waste dumps; species richness, heavy metals.

2. Introduction

Solid wastes are unwanted materials that are more of anthropogenic than natural origin. They consist of day-to-day consumed and discarded items such as food wastes, containers, textiles, product packaging and other numerous wastes from residential, commercial, electronics, institutions and industries (Singh et al. 2011; Amos-Tautau et al. 2014; Oketola and Akpotu 2015). The continuous accumulation of the municipal solid waste (MSW) is associated with heavy metals (HM) generation which are common in the urban centers where accelerated industrial, agricultural, and intensive economic activities prevail. However, metals in small quantities are needed by plants and animals for survival but, they became toxic to the ecosystem when in higher concentrations (Alloway 2004; Kabata-Pendias 2010; Ogbonna et al. 2013; Ali et al. 2013; Liang et al. 2016). For instance, the HM from the MSW was reported to have decreased the soil microbial activities which consequently influenced the concentrations of the essential nutrients' availability for plants' uptake in Nigeria (Ogbonna et al. 2013; Oketola and Akpotu 2015), and in India (Behera et al. 2013). The disposal and management of MSW is a challenging issue in developing countries because of the associated ecological, social and economic reasons (Oketola and Akpotu 2015; Obiora et al. 2016). Open MSW dumps are generally unhygienic environment where disease-carrying vermins such as flies, insects, snakes and rats proliferate, and their toxicity is intensified by their proximity to the motor-ways (Wheeler and Rolfe 1979; Rodriguez-Flores and Rodriguez-Castellon 1982; Oketola and Akpotu 2015). The fluids that ooze and seep from the waste heaps enter and contaminate soil (Monaci et al. 2000; Liu et al. 2015; Oketola and Akpotu 2015), surface and underground water (Alloway 2004; Khan et al. 2008; Onajake and Frank 2012; Ogbonna et al. 2013) and plants (Anikwe and Nwobodo 2002; Ali et al. 2013; Ogbonna et al. 2013; Xue et al. 2013; Li et al. 2016) while, methane and other toxic gases are discharged into the surrounding air as the micro-organisms decompose the wastes (Ali et al. 2014).

The combination of MSW and the vehicle-induced hazardous elements such as Cr, Pb, Cd, and Zn have compounded the effects of pollutants on the soil and plants along the roadsides. Several studies for example, have reported the effects of metal contamination on the soil and plant from waste dumps (Alloway 2004; Barbaferi et al. 2011; McBridge et al. 2014), and toxic gasses from the roads-side heavy traffic (Ferretti et al. 2006; Green et al. 2008; Gupta et al. 2011). In Nigeria, proliferation of Solid waste especially along the major motorways has recently been one of the three major environmental and social issues, including flooding, militancy and desertification (Ayuba et al, 2013). Heavy metals such as Cr, Pb, Zn, Ba and Cd are common soil and vegetation contaminants, but this study focused on the effects of Cr, Pb and Zn as the potential toxic elements (PTE). Many authors within and outside the same area have investigated soil-vegetation responses to Cr (Gupta et al. 2011; Xue et al. 2013; Amos-Tautau et al. 2014; Liu et al. 2015; Liang et al. 2016), Pb (Ferretti et al. 2006; McBridge et al. 2014; Wu et al. 2016) and Zn (Anikwe and Nwobodo 2002; Ogbonna et al. 2013; Li et al. 2016; Obiora et al. 2016) yet, it is rare to find studies which evaluated the combined impacts of Cr, Pb and Zn on the urban soil, plant and species richness and compositions in relation to road-side waste dumps.

We hypothesized that in addition to the significant effects on the soil and plant chemical properties, the MSW dumps affect the species composition and richness. The study aimed at investigating the impacts of the HM caused by MSW dumps on soil and plant chemical properties at the dump sites along the main roads linking major cities in Nigeria. Within this context, the study aimed at answering the following questions:

(i) What are the major effects of Cr, Pb and Zn on the soil chemical properties?

(ii) What are the contents of the heavy metals in the plants?

(iii) Do the species compositions and richness differ significantly across the monitored locations?

(iv) Which species or parts of the plants were more tolerant or vulnerable to the contaminated soil?

2.1. Materials and methods

Study Area

The research was conducted in Nigeria, a developing country in West Africa located between latitude 4^0N to14^0N and longitude 3^0E to 15^0E with a land area of 923,768km^2 and over 50 major roads and nodes connecting the major cities. Nigeria has lowland and highland areas. The Niger-Delta (Coastal plain) region formed the lowest topography (0 to 100m) while the highest altitude is Adamawa Mountain (1800m to 2400m) with Jos Plateau (200m to 1500m) as the coldest place. There are typically two different climate seasons such as wet season (March to early November) and dry season with Harmattan (late November to February). Temperature ranges from 23^0C to 40^0C (from the South to the Northern part). Average annual rainfall ranges from 500mm-3000mm (from the North to the South). There are six vegetation types namely; mangrove, Fresh-water swamp, Rainforest, Guinea Savanna, Sudan Savanna and Sahel Savanna which are grouped into three major agroecological zones such as Forest, Savanna and Montane/Highland Vegetation (Iwena 2000). The soils are of loamy-sand properties and primarily of Luvisols, Vertisols Lithosols Regosols and Ferralsols taxonomical groups formed from carbonaceous shale weathered from sedimentary and metamorphic rocks (Jagtap 1995) which were lately generalized under chromic cambisols and ultisols after the FAO system of soil classification (FAO 2001).

Description of the dump sites

The dump sites were between 15-20 years old, and 3-8 hectares in area. The dumpsites are located in relatively plain land with about 60-90 meters above sea level. The open dumps contain 7 to 11 tons of unsorted municipal wastes mainly made up of agricultural, household, automobile, and industrial effluents including leaves and food remnants, paper, rags, plastic and polyethene, tins. Other are metals, bottles, glasses, laboratory wastes, junked car parts and a variety of miscellaneous materials which are dumped daily at each site. The dominant vegetation at the dump sites were shrubs (*Clausena suffruticosa*, *Bryophyllum pinnatum)*, herbs (*Celosia cristata, Crotalaria pallid, Chromolaena odorata, Curcuma longa)*, grasses *(Andropogon gayanus, Brachiaria decumbens, Panicum maximum),* legumes species *(Leucaena leucocephala, Cajanus cajan), and* forbs *(Vernonia colorata, Anthemis tinctoria and Vernonia nigritiana).* Tree species are rarely found at the dump sites because the dump sites are on regular occasions burnt and the trees are either cut for local tent/shelter or used as firewood and are often prevented from regeneration in contrary to the other plant species which regrow within a shorter time.

Experimental Design, Data collection and sampling

Five MSW dump sites were selected based on a reconnaissance survey. The existing literature and record on the anthropogenic activities that generated the solid wastes in the chosen locations were also used. The locations with the highest records of socioeconomic activities and dump sites were identified (Appendix Figure 1). The five locations sampled were Sokoto-Zaria, Enugu-Onitsha, Ibadan-Lagos, Jos-Abuja, and Port Harcourt-Warri. At each of these locations, a dump site and a dump-free uncultivated land (control site) were chosen at correspondingly 100 meters apart. At each dump sites, four points were identified for the collection of plant and soil samples. The plant samples were collected through species counting and cutting while, soil samples were collected by digging four profile pits. Similarly, four pits (control) were created and sampled as done at the dump sites. This summed up to 20 sampling points: 10 for the dump sites and 10 for the control sites.

Table 1. Cover (in %) of shrubs, herbs, grasses, legumes and forbs and cover of the most abundant species in each functional group. Numbers represent mean values in dump sites and control sites for all the experimental locations

Species	Dump site	Control site	Species	Dump site	Control site
Total cover of Shrubs	**17.4**	**26.5**	**Total Cover of Grasses**	**16.5**	**21.9**
Bryophyllum pinnatum	2.2	2.7	*Andropogon gayanus*	2.7	3.1
Campsis radicans	1.7	2.5	*Brachiaria decumbens*	2.9	3.6
Celosia cristata	2	2.4	*Cenchrus ciliaris*	1.1	1.9
Clausena suffruticosa	2.2	3.1	*Cynodon dactylon*	1.6	2.5
Dalbergia stipulacea	1.3	2.1	*Diplazium esculentum*	1	1.2
Flemingia stricta	0.6	1.4	*Imperata cylindrica*	0.8	0.9
Sida acuta	0.5	0.7	*Panicum maximum*	1.1	1.4
Ixora cuneifolia	0	0.1	*Axonopus compressus*	1.5	1.8
Jatropha gossypifolia	0.4	1.1	*Poa pratensis*	2.1	3.1
Leea aequata	0.5	0.8	*Panicum purpureum*	1.6	1.8
Melastoma malabathricum	1.3	2.2	*Tripsacum laxum*	0.1	0.6
Sansevieria roxburghiana	1.6	2.7	**Total Cover of Legumes**	**13.7**	**16**
Senna alata	2.1	3.6	*Cajanus cajan*	2	2.1
Smilax laurifolia	1	1.1	*Centrosema pubescens*	1.3	1.8
Total cover of Herbs	**18.2**	**25.1**	*Gliricidia sepium*	0	0.3
Euphorbia hirta	1.3	1.6	*Lablab purpreus*	0.4	0.6
Amaranthus spinosus L.	0.8	0.9	*Leucaena leucocephala*	1	1.1
Solena amplexicaulis	1.4	2.1	*Marotyloma uniflorum*	1.3	1
Centella asiatica	0	0.6	*Calopogonium mucunoides*	1.8	2
Chromolaena odorata	2.5	1.8	*S. guianensis (Schofield)*	0.9	1
Cissus repens	1.4	2.4	*Stylosanthes guianensis (cook)*	2.4	3
Commelina diffusa	1.7	2.5	*Stylosanthes hamata (Verano)*	1.1	1.3
Crotalaria pallida	2.2	3.8	*Stylosanthes humilis*	1.5	1.8
Curcuma longa	2.1	2.7	**Total cover of Forbs**	**0.8**	**2.8**
Emilia sonchifolia	0	0.8	*Anthemis tinctoria (cota)*	0.3	1.2
Mikania cordata	2.4	2.6	*Saintpaulia teitensis*	0.2	0.4
Mimosa pudica	1.4	1.9	*Vernonia bamendae*	0	0.1
Ocimum suave	1	1.1	*Vernonia colorata*	0.2	0
Stephania japonica	0	0.3	*Vernonia nigritiana*	0.1	1.1
			Others	33.4	7.7

Soil sampling and chemical properties analysis

At the same points where the major plants (Table 1) were sampled, four points were identified for the collection of soil samples as earlier stated. After clearing the overlying surface wastes, soil samples were collected from the dump sites at 50 cm from the waste heaps using a terrax and terra-scoop in combination of a Dutch soil auger and a spatula. The four profile holes measuring 50cm x 30cm x 50cm (length x depth x width) were dug at the specified 50 cm round the dumpsite, and this was performed at each of the five MSW dump sites. The soil samples were collected at every 5 cm depth and mixed together. The samples were air dried for four days while the visible pebbles, biomass residues, roots, and other organic debris were removed. These procedures and measurements were also followed when collecting soil samples at the control sites. The soil samples were ground and sieved through a 2 mm sieve and stored in labelled polythene bags before taken to accredited laboratory of the department of soil and crop sciences for analysis. The concentration of the heavy metals (Cr, Pb, Zn,) and essential nutrients (N, P, K) were measured. To extract Plant-available P and K, the Mehlich III was used (Mehlich 1984) and then determined by inductively coupled plasma–optical emission spectrometry (ICP–OES). pH (in water) was determined by the method of McLean (1982), while total N was analyzed according to the Macro-Kjeldahl method (Bremmer 1965). On the other hand, the contents of the heavy metals (Cr, Pb, Zn) in the soil samples were determined following the Clayton and Tiller (1979) method. The concentrations were further analyzed using ICP-OES analyzer (720 Series, Agilent Technologies). However, several HM were measured but Cr, Pb, and Zn were the focus of the study because they were the most concentrated in the area. The mean of four sub-samples from each monitored site was used for statistical analyses.

Table 2. Correlation between soil HM (Cr, Pb, Zn) and nutrients (N, P, K) in dump sites and control sites

	Cr_dump	Pb_dump	Zn_dump	Cr_control	Pb_control	Zn_control	N_dump	P_dump	K_dump	N_control	P_control	K_control
Cr_dump	1											
Pb_dump	0.63	1										
Zn_dump	**0.62***	**0.92***	1									
Cr_control	0.05	-0.01	0.65	1								
Pb_control	-0.01	-0.24	0.11	**-0.67***	1							
Zn_control	-0.09	-0.02	0.29	**0.71***	**0.88***	1						
N_dump	0.08	0.04	-0.19	-0.45	0.14	-0.64	1					
P_dump	0.02	0.16	**0.66***	-0.42	0.49	-0.51	**0.80***	1				
K_dump	0.14	0.47	**0.58***	-0.03	0.06	0.36	0.32	0.37	1			
N_control	-0.27	-0.06	0.09	0.57	-0.37	**0.69***	-0.23	0.12	-0.03	1		
P_control	-0.15	0.01	-0.43	-0.35	-0.08	**0.61***	-0.38	-0.04	0.01	0.53	1	
K_control	-0.21	-0.07	-0.08	0.003	-0.69	**0.73***	-0.56	-0.48	-0.37	**0.91***	0.26	1

Note: Figures in **bold** and asterisk (*) were significantly correlated at $p < 0.05$.

Plant sampling and chemical properties analysis

Vegetation samples were collected before the soil samples though at the same areas. Before cutting the plants, their abundance was first recorded at each site using quadrat method (Kent and Coker 1992) while, the species richness (number of species per m^2) was determined by surveying and taking inventory through counting of all visible species. The cutting was done manually at a stubble height of 5cm using cutlass. The biomass samples were collected after species sampling/counting. The plant Samples were weighed, oven dried for 2 days at 85 ^{0}C until totally exsiccated and then weighed again. The dried plant material was ground in a ball mill to a fine powder (Chu et al. 2004). The plants sampled included 14 species each from shrubs and herbs, 11 species each from grasses and legumes and 5 forbs species. The most abundance species in the list for each functional group are shown in Table 1. All the samples were put into appropriately labeled polyethylene bags and taken to the laboratory for analysis. Both the plant and soil samplings were performed in July which coincides with the vegetation growing season, heavy waste dumbing and decomposing processes. Plants concentrations of minerals (N, P, K), heavy metals (Cr, Pb, Zn) and the dry matter production were determined from the aboveground biomass samples which were used for analysis after digestion in aqua regia by applying some of the most suitable methods used for soil samples analysis.

Data and statistical analysis

The soil and plant data were analyzed using one-way ANOVA followed by a post hoc Comparison-Tukey HSD test. These were applied to evaluate the impacts of the waste dumps on the soil and plant chemical properties. The use of ANOVA was permitted as all the required assumptions including normality were achieved. The relationships between soil and plant chemical properties (under dump and control sites), sampling area and species richness were analyzed by linear regression analysis including the coefficient of alienation ($\sqrt{1-R^2}$) which measures the degree of un-relatedness as described by Steel and Torrie (1980). All the regression analyses were performed using the IBM SPSS 20.0 (IBM Corp 2011).

The soil to plant metal transfer was determined as transfer factor (TF) using the equation as applied by Obiora et al, (2016):

$TF = C_{plant} / C_{soil}$

Where C_{plant} represents the heavy metal concentration in the plants as extracted whereas, C_{soil} stands for the heavy metal contents in the soil.

Furthermore, a redundancy analysis (RDA) followed by the Monte Carlo permutation test in CANOCO was introduced to show the relationships between the distribution of elemental concentrations and plant species. The RDA analysis was conducted using CANOCO 5.0 program (Šmilauer and Lepš, 2014), and each analysis was performed with 999 permutations. Species data were log-transformed ($y' = \log_{10}(y + 1)$). Ordination figures designed in the CanoDraw program were used to visualize the results of the analyses.

2.2. Result and discussion

Soil chemical properties

The relationships between the soil HM (Cr, Pb, Zn), and nutrients (N, P, K) at the dump sites and control sites are shown in Table 2. At the dump sites, the mean concentration of Zn showed a positively significant correlation with Cr, P and K concentrations, and negatively correlated with N concentrations. Lack of N_2 - fixing plants because of the HM could be attributed to the negative correlation between Cr and N concentrations (Obiora et al. 2016). Although, the Zn concentrations might have increased P and K contents yet, their availability for the plants' uptake was hindered by the high concentration of Cr and Pb. On the other hand, at the control sites, the mean contents of Cr indicated strong relationships with Zn and Pb concentrations. Similarly, the mean concentrations of Zn were significantly correlated with the mean concentrations of N, P, and K at the control sites. The strong relationship could be explained by the reason that the elements originated from the same source (Ogbonna et al. 2013; Oketola and Akpotu 2015). The study also discovered a significant relationship between soil Zn and Pb concentrations at both dump sites and control sites. And this could be explained by the coherent report by Alloway et al (1990) that Zn-rich soils (0.1 -1.0% Zn) often contain high contents of Pb.

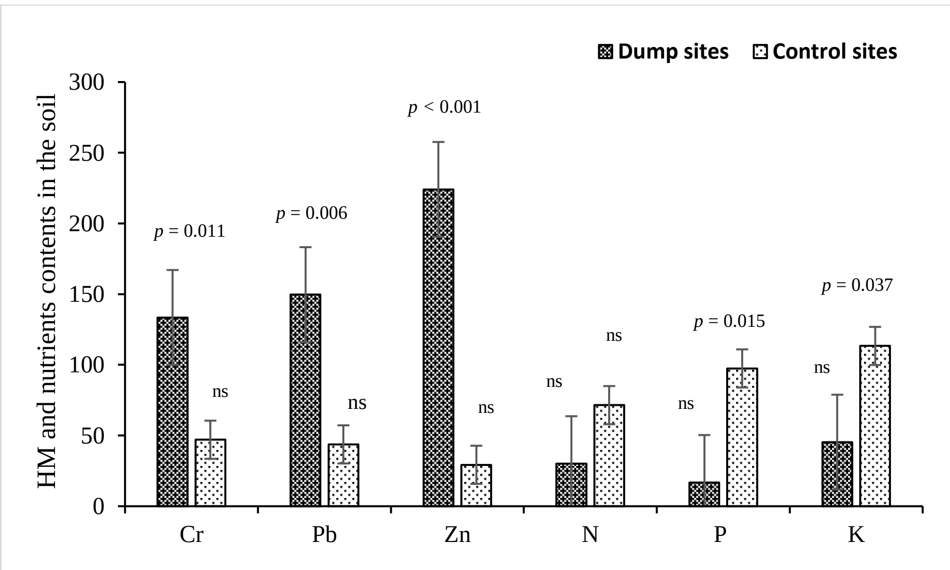

Figure 1. Mean HM (Cr, Pb, Zn), and mean nutrients (N, P, K) concentrations (in mg kg^{-1}) in the soil under the dump sites and the control sites in the study area. Significant differences between the dump sites and control sites in relation to the different sampling locations calculated by one-way ANOVA analyses are significant at $p < 0.05$. Values are means of four replicates. Error bars show standard error (SE) of the mean differences. ns = results of ANOVA analyses were not significant.

Typically, there was no significant relationships found between the soil HM contents at the dump sites and the soil HM contents at the control sites; neither was there any relationship between the soil nutrients at the dump sites and control sites. The differences in the sources of the contaminants might be responsible for the none relationships (Ogunfowokan et al. 2009; Oketola and Akpotu

2015). This finding was further supported by the result of the coefficient of un-relatedness (Table 3).

Table 3. Relationships between the contents of soil and plant chemical properties in the dump sites and control sites

		Coefficient of alienation ($\sqrt{1-R^2}$)*	*p*-value
Soil	Cr (mg kg^{-1})	0.92	< 0.001
	Pb (mg kg^{-1})	0.87	< 0.001
	Zn (mg kg^{-1})	0.74	< 0.001
	N (mg kg^{-1})	0.42	0.02
	P (mg kg^{-1})	0.94	0.007
	K (mg kg^{-1})	0.77	0.015
	pH	0.02	0.041
Plant	Cr (mg kg^{-1})	0.91	< 0.001
	Pb (mg kg^{-1})	0.89	< 0.001
	Zn (mg kg^{-1})	0.96	< 0.001
	N (mg kg^{-1})	0.53	0.013
	P (mg kg^{-1})	0.88	< 0.001
	K (mg kg^{-1})	0.81	< 0.001

* that is degree of un-relatedness

The result of the mean concentrations of HM and nutrients in soil under the dump sites and control sites is shown in Figure 1. The mean content of Cr (133.5 mg kg^{-1}), Pb (149.67 mg kg^{-1}), and Zn (224.07 mg kg^{-1}) at the dump sites were not only about thrice higher when compared with control sites but were also significant (Cr ($p = 0.011$), Pb ($p = 0.006$), and Zn ($p < 0.001$)). On the other hand, at the control sites, the mean concentrations of P ($p = 0.015$), and K ($p = 0.037$) indicated significant differences whereas, the mean concentration of N was not significant. The effect of Cr which prevented the growth of the N_2 fixing plants at the dump sites, and atmospheric deposition from the automobiles at the control sites might be responsible.

The soil contents of Cr and Pb at the dump sites were far above the permissible limits for agricultural soil (Figure 1). For example, the EUC (2006) recommended values for Cr and Pb was 100 mg kg^{-1} while, CCME (2007) recommended 64 mg kg^{-1} for Cr and 70 mg kg^{-1} for Pb. However, our result was also higher than the data from Taiyuan City of China (Liu et al. 2015) but in agreement with the reports from Southern Nigeria (Obiora et al. 2016), Beijing-China (Khan et al. 2013), India (Singh et al, 2010), Pakistan (Mahmoud and Malik 2014), and Ghana (Musah et al, 2013). Similarly, the Zn soil content was higher above the CCME (2007) permissible limit of 200 mg kg^{-1} for agricultural soils but were within the range of 300 mg kg^{-1} proffered by EUC (2006). The Zn soil content was relatively high at the dump sites when compared to the result from same area where Zn concentration ranged from 62.2 mg kg^{-1} to 68.5 mg kg-1 (Anikwe and Nwobodo 2002). As essential element for higher plants, Zn is phytotoxic at elevated contents and this consequently had adverse effects on the soil fertility and plant growth (Alloway et al. 1990). Generally, the study revealed that the dump sites had higher concentrations of HM in the soil as compared with the control sites: the solid wastes, metal mobilization and/or immobilization by soil components might be responsible (Brunetti et al. 2009).

Table 4. Mean heavy metals transfer factor (HMTF) for different vegetation types in the study area

Vegetation Types	Cr	Pb	Zn
Shrubs	0.49	0.17	0.38
Herbs	0.11	0.09	0.27
Legumes	0.08	0.22	0.19
Forbs	0.02	0.01	0.46
Grasses	<0.01	0.18	0.01

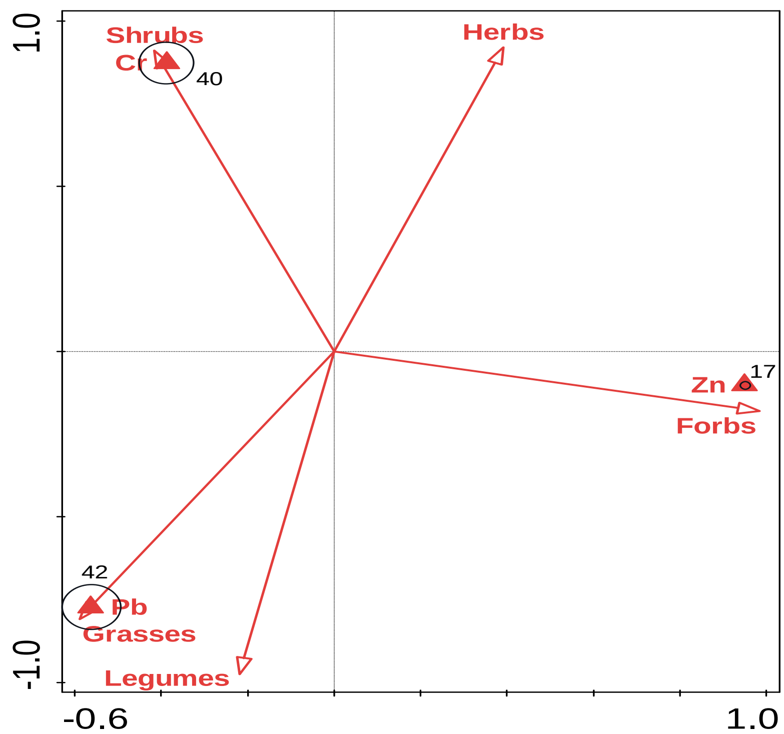

Figure 2. Distribution of the functional groups of the plant species in relation to the heavy metals.

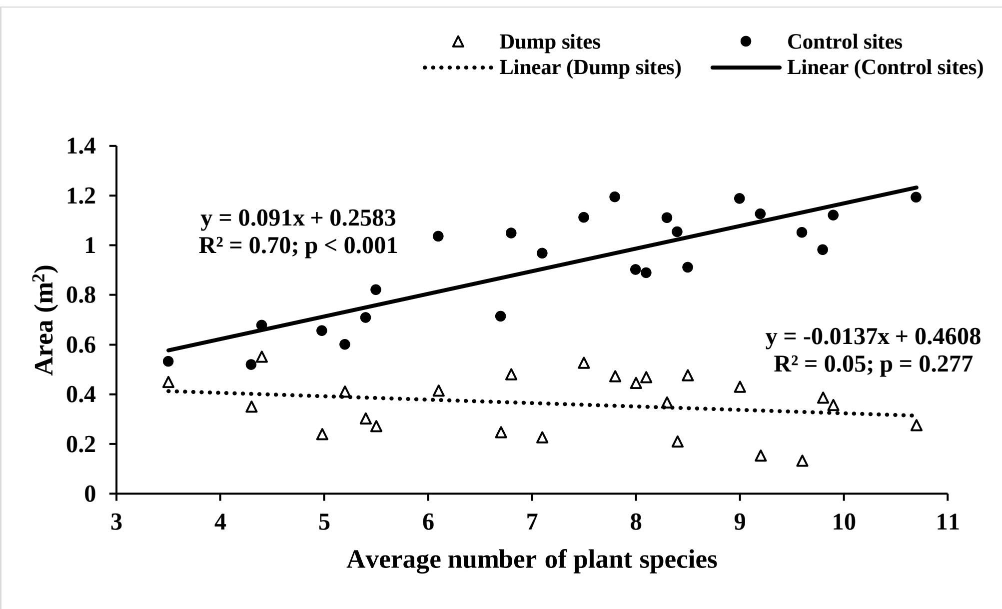

Figure 3. Relationships between the mean values of plant species richness and the area in the dump sites and control sites for the different locations investigated.

Table 5. Mean concentrations (in mg kg^{-1}) of heavy metals (Cr, Pb, Zn) in plants species parts under the dump sites and control sites

	Dump sites									Control sites								
Species	Leaves			Shoots			Roots/tubers			Leaves			Shoots			Roots/tubers		
	Cr	Pb	Zn	Cr	Pb	Zn	Cr	Pb	Zn	Cr	Pb	Zn	Cr	Pb	Zn	Cr	Pb	Zn
B.*pinnatu*	37±3	15±4	56±22	15±2	11±2	11±4	5±0.1	3±0.2	7±0.4	6±0.9	3±0.7	9±2	1±0.4	1±0.1	3±0.7	0.4±0.1	0.3±0	0.8±0.1
C.*suffrutic*	57±9	34±11	67±23	21±6	10±3	6±3	4±1	1±0.5	5±0.8	13±2	10±3	28±9	7±1	4±1	15±4	2±0.5	1±0.3	4±2
C.*cristata*	46±12	30±9	79±36	20±6	10±2	14±5	5±2	3±1	6±2	20±5	16±3	33±17	9±2	3±1	13±7	2±1	2±0.6	7±3
M.*cordata*	11±3	21±2	90±1	5±0.5	5±1	32±13	3±1	2±0.7	4±1	5±2	14±5	36±17	1±0.6	5±2	18±6	0	1±0	5±2
M.*pudica*	11±6	16±7	83±5	5±0.9	8±3	40±13	3±0.8	7±3	13±5	3±0.2	2±0.1	21±4	1±0.7	0.4±0	8±3	0	0	2±0.3
C.*pallida*	10±7	7±3	88±21	0.6±0.3	4±1	38±10	2±0.4	1±0.1	8±4	2±1	1±0.8	19±3	0.8±0.3	0.5±0.1	6±4	0	0	3±1
B.*decumb*	10±2	8±2	2±0.5	1±0.8	1±0.7	0.1±0.2	0.3±0.1	0.5±0.3	0.1±0	1±0.9	1±0.4	2±0.7	0.9±0.2	0.6±0.5	1±0.4	0.4±0	0	0.8±0
A.*gayanus*	10±5	11±7	1±1	0.7±0.4	0.9±0.2	0.1±0	0.9±0.4	0.2±0.1	1±0.5	2±1	2±0	0.7±0	1±0.4	1±0.5	0.4±0	0.3±0	0.2±0.1	0
P.*maximu*	13±3	10±4	2±1	0.1±0	2.1±0.7	0.1±0	0	0.4±0.1	0	3±1	3±0	0.5±0	1±0.9	1±1	0.9±0	0	0	0
C.*cajan*	60±24	35±21	93±32	32±17	21±13	16±0.9	10±1	6±2	8±5	1±0.8	0.4±0	3±0.1	0	0	0.6±0.3	0	0	0
L.*leucoce*	8±2	12±5	18±6	2±0.4	1±0.7	3±1	1±0.5	0.6±0.3	1±1	1±0.7	2±1	5±2	0	0.4±0	2±1	0	0	0
C. odorata	65±27	49±16	99±46	38±12	23±5	41±14	13±3	7±2	19±8	9±2	6±3	17±9	2±0.8	1±0	5±0.6	1±0.9	1±0	2±0.9
V.*colorata*	5±4	2±1	2±0.6	0.1±0	1±0.5	0.1±0	0.3±0.2	0.5±0.1	0.8±0.4	1±0.1	0.8±0.3	1±0.5	0.4±0	0	0.7±0	0	0	0
V.*nigritia*	5±2	3±1	4±3	0.7±0.3	1±0.8	0.1±0	0	0	0.6±0.1	1±0.2	1±0	1±0.9	0.3±0	0	0.4±0	0	0	0
I.*cylindric*	14±4	6±5	29±8	5±2	4±0.9	11±5	2±1	1±0.4	5±3	3±2	1±0.5	8±3	5±1	1±0.8	11±3	0.7±0.1	0	2±0.4

Plants chemical contents, compositions, and richness

The dominant plant species in the study area are shown in Table 1. Shrubs, herbs, legumes, grasses, and forbs were the common species with shrubs and herbs having the highest percentage coverage of 17.4 and 18.2 at the dump sites; and 26.5 and 25.1 at the control sites respectively. The vegetation types responded differently to the effects of the HM. The higher concentrations of each metal in a particular plant is an indication that such plant has more tolerance for such metal. For instance, shrubs were more related to Cr, and forbs were more tolerant with Zn while, Pb was highest in legumes and grasses (Table 4 and Figure 2). The mean HM transfer factor revealed that Zn had the highest metal transfer factor from soil to plant while, shrubs and herbs showed the highest concentrations of the metals which developed an order of shrubs > herbs > legumes > grasses > forbs. This result was consistent with the findings of Obiora et al, (2016); and might be explained by the reason that the shrubs and herbs are perennial with higher tolerance to the metals as compared with the grasses and forbs (Khan et al. 2010). However, the high contents of the HM found in *Cajanus cajan* and *Chromolaena odorata* was remarkable. The high tolerance of the metals by *C. cajan* and *C. odorata* could probably be a good reason for the high content of metals they absorb and making them locally useful for biophytoremediation (Aprill and Sim 1990; Bada and Olarinre 2012; Ismail et al. 2014).

The amount of metals absorbance by plants into various parts of their tissues differs. In this study, the mean concentrations of the HM in different plants' segments are shown in Table 5. Kabata-Pendisa (2000) reported that the rates of metal movement in the plant tissues varies based on plant type, age, location, and elements involved. Our study revealed that the leaves of the plants had the highest concentrations of the metals while, the roots/tubers had the lowest concentrations producing the order: leaves > shoots > roots/tubers.

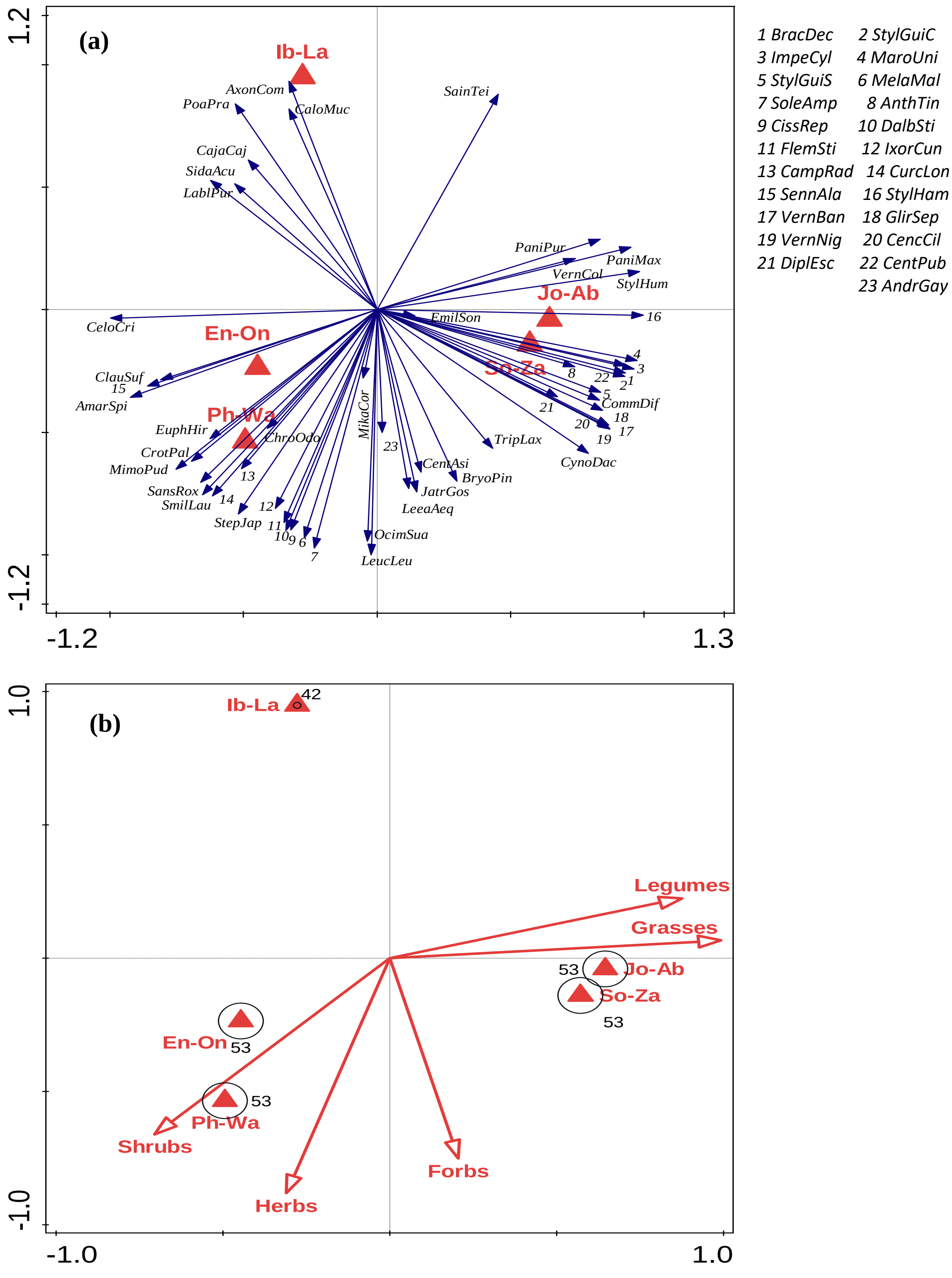

Figure 4. Ordination diagram showing the results of RDA analysis of: (a) plant species composition; (b) abundance and distribution of the functional groups of the plant species in relation to the different investigated locations in 2015. The location abbreviations, So-Za, En-On, Ib-La, Jo-Ab, Ph-Wa represent Sokoto-Zaria, Enugu-Onitsha, Ibadan-Lagos, Jos-Abuja, and Port Harcourt-Warri respectively. Species abbreviations are as follows: *BryoPin: Bryophyllum pinnatum, CampRad: Campsis radicans, CeloCri:Celosia cristata, ClauSuf: Clausena*

suffruticosa, DalbSti: Dalbergia stipulacea, FlemStr: Flemingia stricta, SidaAcu: Sida acuta, IxorCun: Ixora cuneifolia, JatrGos: Jatropha gossypifolia, LeeaAeq: Leea aequata, MelaMal: Melastoma malabathricum, SansRox: Sansevieria roxburghiana, SennAla: Senna alata, SmilLau: Smilax laurifolia, EuphHir: Euphorbia hirta, AmarSpi: Amaranthus spinosus, SoleAmp: Solena amplexicaulis, CentAsi: Centella asiatica, ChroOdo: Chromolaena odorata, CissRep: Cissus repens, CommDif: Commelina diffusa, CrotPal: Crotalaria pallida, CurcLon: Curcuma longa, EmilSon: Emilia sonchifolia, MikaCor: Mikania cordata, MimoPud: Mimosa pudica, OcimSua: Ocimum suave, StepJap: Stephania japonica, AndrGay: Andropogon gayanus, BracDec: Brachiaria decumbens, CencCil: Cenchrus ciliaris, CynoDac: Cynodon dactylon, DiplEsc: Diplazium esculentum, ImpeCyl: Imperata cylindrical, PaniMax: Panicum maximum, AxonCom: Axonopus compressus, PoaPra: Poa pratensis, PaniPur: Panicum purpureum, TripLax: Tripsacum laxum, CajaCaj: Cajanus cajan, CentPub: Centrosema pubescens, GlirSep: Gliricidia sepium, LablPur: Lablab purpreus, LeucLeu: Leucaena leucocephala, MaroUni: Marotyloma uniflorum, CaloMuc: Calopogonium mucunoides, StylGuiS: Stylosanthes guianensis (Schofield), StylGuiC: Stylosanthes guianensis (cook), StylHamV: Stylosanthes hamata (Verano), StylHum: Stylosanthes humilis , AnthTinC: Anthemis tinctoria (cota), SainTei: Saintpaulia teitensis, VernBam: Vernonia bamendae, VernCol: Vernonia colorata, VernNig: Vernonia nigritiana.

This might be attributed to excessive rate of transpiration by the plants to keep their water balance (Tani and Barringston 2005; Lato et al. 2012; Obiora et al. 2016). Another possible reason for the high concentrations of metals in the leafy parts of the vegetables could be explained by the particulate contaminants of plants from the dumps and/or vehicles aerosols which might be more important than the plants' uptake via their roots (Ferretti et al. 2006; Mosbaek et al. 2009; Ogunfowokan et al. 2009). Other work in support of this finding was conducted at the Ife University waste dump site-Nigeria, where *T. triangulare* and *A. esculentus* were reported to have increased Pb concentrations in their leaves compared to other parts of the plants (Amusan et al. 1999). Similarly, the effects of HM on peppermint and cornmint was examined by Amusan et al, (1999), and it was concluded that the Zn concentration was greater in plants leaves than either the shoots or roots.

The species richness in relation to the area was also measured and the result is shown in Figure 3. At the control sites, the number of recorded plant species increased with area leading to a positively significant relationship ($p < 0.001$; $R^2 = 0.70$). On the other hand, the relationship between number of species and sample area was not significant under the dump sites (Figure 3).

Locational differences

The redundancy analysis (RDA) showed significant ($p < 0.001$) differences for the first ordination axis and all ordination axes in species compositions and sampling locations (Figure 4a). The percentages of explained variability by the first ordination axis and all ordination axes were 55.1% and 45.6% respectively. The species richness tends to show three different groups of distributional trend in relation to the locations: the shrubs and herbs mainly associated with En-On and Ph-Wa locations; grasses and legumes abundant at Jo-Ab and So-Za; Ib-La location has mixture of herbs, shrubs, grasses and legumes with no forbs (Figure 4b). More than 80% of the species are found at the Ph-Wa, En-On, Jo-Ab and So-Za locations while, Ib-La had lower percentage. The higher concentrations of soil pH and HM at the Ib-La locations (Appendix Table 1) was probably the cause of lower species percentage as compared with other locations.

2.3. Conclusion

The long periods of continuous dumping of MSW coupled with the discharge from the motorists caused elevated concentrations of the HM (Cr, Pb and Zn) at the dump sites which had substantial effects on the soil and vegetation. The shrubs and herbs showed higher metals concentration and tolerance in comparison with the legumes or forbs because the shrubs and herbs perennial plants while, the forbs and legumes were primarily annual plants. The plants leave had higher contents of the HM as compared with either the shoots, roots or tubers, and this could be caused by the high temperature in the area which requires the plants to keep their water balance through excess transpiration. This processes consequently transport more toxic substances to the leafy parts. In contrast to the dump sites, species richness increased with area at the control sites. Dump sites have in most cases been reported to have high contents of the macro and micro-nutrients due to high pH and organic matter but on the contrary, our finding was different making the study important and at the same time requires a long-term monitoring to validate the result. Further study on the effects of the HM on soil and plant is necessary at dump sites located far from the roads as to ascertain if aerosols from automobiles contributed substantially to the soil pollution. More so, the government and individuals mostly affected should adopt proper management system to dispose the wastes while, recycling and bio-phytoremediation processes are also recommended.

Acknowledgement

We give thanks to the Federal Environmental Protection Agency (FEPA) officers who helped in the provision of preliminary information on dump sites. The effort of the National Road Transport Workers (NURTW) of Nigeria is appreciated. The intellectual support of the journal's peer blind reviewers whose remarks made this paper a success is also acknowledged. We also acknowledge The Czech University of Life Sciences, Prague for their support to travel to Nigeria and conduct the research.

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Appendix Table 1

Mean concentrations of soil and plant aboveground biomass (AGB) of heavy metals (Cr, Pb, Zn) and Ph values for the dump sites and the control sites in the investigated locations. *F*-ratio = *F*-statistics for the test of a particular analysis; *P*-value represents corresponding probability value from the one-way ANOVA. Numbers represent the average of four replicates; ± standard error of the mean (SE); Significance differences (p < 0.05) between investigated locations/sites in relation to the soil elements in accordance with the Tukey's post hoc test are shown by different letters (a > b > c > d > e) in the row. Sampling locations were Sokoto-Zaria (So-Za), Enugu-Onitsha (En-On), Ibadan-Lagos (Ib-La), Jos-Abuja (Jo-Ab), PortHarcourt-Warri (Ph-Wa).

SITES			Locations					One-way ANOVA	
	Parameter		So-Za	En-On	Ib-La	Jo-Ab	Ph-Wa	*F*-ratio	*p*-value
DUMP									
	Soil	Cr (mg kg^{-1})	145.64±7.53b	107.01±10.50c	224.63±12.31a	96.05±17.28d	94.18±12.27d	1.86	<0.001
		Pb (mg kg^{-1})	148.46±5.15	150.13±6.52	149.72±6.96	148.72±4.88	151.31±4.17	5.43	0.216
		Zn (mg kg^{-1})	221.51±27.41b	206.11±22.64c	251.23±31.05a	219.33±42.31bc	222.19±32.51b	124	0.007
		pH	6.95±0.15	6.85±0.32	7.16±0.43	6.88±0.57	6.74±0.18	0.52	0.114
CONTROL									
	Soil	Cr (mg kg^{-1})	35.21±5.74c	37.83±8.33c	51.66±10.60a	41.57±9.16b	53.92±11.26a	0.55	0.012
		Pb (mg kg^{-1})	44.51±8.12	45.78±9.90	43.91±11.78	41.80±6.47	42.63±7.53	6.26	0.054
		Zn (mg kg^{-1})	42.67±36.18a	15.27±25.18d	26.48±21.19c	23.02±20.79c	35.11±26.42b	0.91	<0.001
		pH	5.83±1.23	5.87±1.11	6.01±1.38	5.80±1.22	5.84±1.27	3.41	0.375
DUMP									
	Plant AGB	Cr (mg kg^{-1})	183.42±23.01c	224.04±17.76b	222.17±21.60b	157.70±19.42d	272.49±25.00a	11.6	<0.001
		Pb (mg kg^{-1})	88.94±22.13c	157.96±31.45ab	144.53±42.16b	66.32±19.77d	126.17±27.03a	47.3	<0.001
		Zn (mg kg^{-1})	206.88±16.17	208.41±18.42	205.74±20.10	201.31±15.24	206.22±17.54	0.78	0.091
CONTROL									
	Plant AGB	Cr (mg kg^{-1})	27.44±12.00d	41.59±18.29c	94.6±23.62a	26.57±33.15d	87.24±27.98b	24.8	<0.001
		Pb (mg kg^{-1})	67.75±24.35ab	44.37±21.58c	71.91±19.60a	32.67±22.34d	59.92±18.07b	19.6	0.032
		Zn (mg kg^{-1})	81.43±2.67b	52.16±11.73e	60.31±16.27d	71.11±31.52c	92.45±9.44a	35.6	<0.001

Appendix Figure 1. Photograph showing one of the five dump sites investigated for the study

CHAPTER THREE

Soil organic carbon and total nitrogen stocks as affected by different land use in an Ultisol in Imo watershed, Southern Nigeria

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Abstract

Soil organic carbon (SOC) and soil total nitrogen (STN) concentrations and stocks are essential for improving soil quality and increasing C-reservoir. This study aimed at quantifying the dynamics in soil properties under different land uses in Imo watershed where there is no knowledge about the effects of land use on SOC and STN pool. Six land uses: arable land (AL), forest land (FL), grassland (GL), shrubland hills (SL), urban built-up green (UL), and the freshwater swamp and mangrove wetland (WL) were classified using ArcGIS 10.1 and FAO land use classification system. Soil samples were collected and analyzed from each land use under different soil depths and slope positions with three replications. Topsoil layer (0-30cm) contributed to more than 90% of the total soil nutrients. Land use significantly affected SOC content, STN content, and bulk density. SOC and STN concentrations were in the order of FL > WL > GL > SL > UL > AL which revealed the potentials of FL and WL for SOC and STN sequestration. The study provides land users with the information to improve soil quality, conserve C and N stocks for ecological sustainability and climate change mitigation.

Key words: Soil ecology, SOC, STN, land use types, agriculture.

3. Introduction

Climate change caused by increasing levels of CO_2 and other greenhouse gases has been identified as a critical issue of global environmental concern (Yan et al. 2018). This contemporary paradigm of global warming is drawing experts' attention to carbon and nitrogen cycles due to their rapid potentials for atmospheric oxides emission (Jia et al. 2018). Soil has the highest organic carbon stocks in the terrestrial ecosystem which twice exceeds the atmospheric content (Lal 2004), and about three times more than the quantity found in vegetation (Zang 2013). Soil as the largest terrestrial reservoir of carbon (IPCC, 2007) plays a vital role in conserving plant nutrients and ameliorating excess CO_2 emission (Jia et al. 2018). Soil is not only a large C-sink but also a fundamental reservoir of nitrogen that can supply essential nutrients for plant use. Annually, about 4% of soil carbon reserves is released into the atmosphere (Li et al. 2014), while about 6.0 Tg of N_2O (1Tg = 10^{12}g) and 4.2 Tg of N_2O are emitted from natural and agricultural soils respectively (Saikawa et al. 2014). Any infinitesimal change in the soil C or N stocks might cause remarkable impact on the atmospheric carbon dioxide and nitrous oxide concentrations. As a consequence of these facts, conserving the C and N stocks is a healthy process that enhances soil fertility, reduces global warming, and consequently promotes sustainable food security and environmental safety.

Unfortunately, this carbon pool (in the soil) is interrupted by various disturbances that influence its storage capacities. In the terrestrial ecosystems, major threats to SOC and STN stocks have been revealed by several authors to include land use types and changes (Conti et al. 2016; Diwediga et al. 2017; Gelaw et al. 2015; Li and Zhao, 2001; Qiao et al. 2015; Qiu et al. 2015; Qi et al. 2018; Wang et al. 2017; Xiao et al. 2017), soil compositions (Udom and Ogunwole 2015) and urbanization (Liu et al. 2018). Other threats to SOC and STN have been agriculture and management practices (Ding et al 2013; Han et al. 2016; Negasa et al. 2017; Wang et al. 2016), vegetation (Lohbeck et al. 2018), soil depth and bulk density (Li et al. 2017; Negasa et al. 2017); Slope and topographical characteristics (Wang et al. 2017), induced climatic factors (Huang et al. 2018), soil erosion (Daniel et al. 2017), and Landscape position (Zilverberg et al. 2018). Primarily, these studies widely focused on the vertical and horizontal distribution of SOC and STN in various land uses. However, in Imo watershed, there is still wide gap in understanding the dynamics of SOC and STN stocks that are influenced by different land uses such as agriculture. Agriculture in this tropical humid region provides more that 80% of the increasing population with means of livelihood. It is worrisome that many of these farmers engage in intensive subsistence agriculture, yet they have limited knowledge and inadequate resources to conserve the soil and improve fertility.

In the past, the government established several land use and soil management programs [such as River basin development authorities (RBDAs), Anambra-Imo river basin development (AIRBD), Directorate of Food-Roads and Rural Infrastructure (DFRRI), Green revolutions, and National agriculture and environmental development scheme] which were geared towards sustaining the ecosystem. Unfortunately, these projects did not only fail to improve soil quality but have ceased to function since the past two decades. Therefore, with the expanding population demand for more food coupled with the risks of global warming, there is urgent need to restore and conserve the soil (Nwaogu et al. 2017). The knowledge of SOC and STN concentrations, stocks, and nutrient cycling in this watershed is indispensable for improving the soil quality, food production and mitigating carbon emissions. Imo river basin as a highly agricultural watershed is threatened by severe changes in land use-landcover. The failure of government schemes, lack of studies and dearth of information have hindered sustainable restoration of soil quality in this region. Based on this background, it is hypothesized that in the Imo watershed and catchments: (1) SOC and STN concentrations and stocks are significantly affected by slope position, soil bulk density and depth; (2) different land use types varied in their potentials for SOC and STN sequestration. These hypotheses were developed as to provide relevant information for policy-makers and land managers for better land management decisions. To measure these hypotheses and fulfil the goals of the study, SOC and STN concentrations and stocks of soils in six land use types (AL, FL, GL, SL, UL, and WL) were tested under two soil depths (0-30cm, 30-60cm), and in three different slope positions (upper, middle and lower) and their results were compared. The study specifically aimed at: quantifying the storage capacity of SOC and STN in different land uses, soil depth, bulk density and slope position.

3.1. Materials and methods

Research area

The study was conducted in Imo watershed located in the south-east and south-south regions of Nigeria (Fig. 1). Geographically the watershed extends between 4° 50' 00''N to 6° 02' 00''N latitude and 6° 04'10''E to 7°34' 15''E longitude, having an area of 4321.4 km^2, and an elevation ranging from about 52 m - 340 m above sea level (m.a.s.l.). The watershed is a humid tropical climate. The mean annual temperature is 28.5° C, while the mean annual rainfall is 2400 mm with over 70% falling between the months of May and October (FORMECU 1998). The soils are classified as Ultisol (Arenic Kandiustult) (WRB 2006) which were developed from sedimentary Ameki Formation and Imo Shale, and unconsolidated coastal plain sands and alluvium of the Niger Deltaic (Amangabara 2015). It has soil pH which ranged from 5.32 to 6.44 with sand, loamy-sand textural characterizations (Udom and Ogunwole 2015).

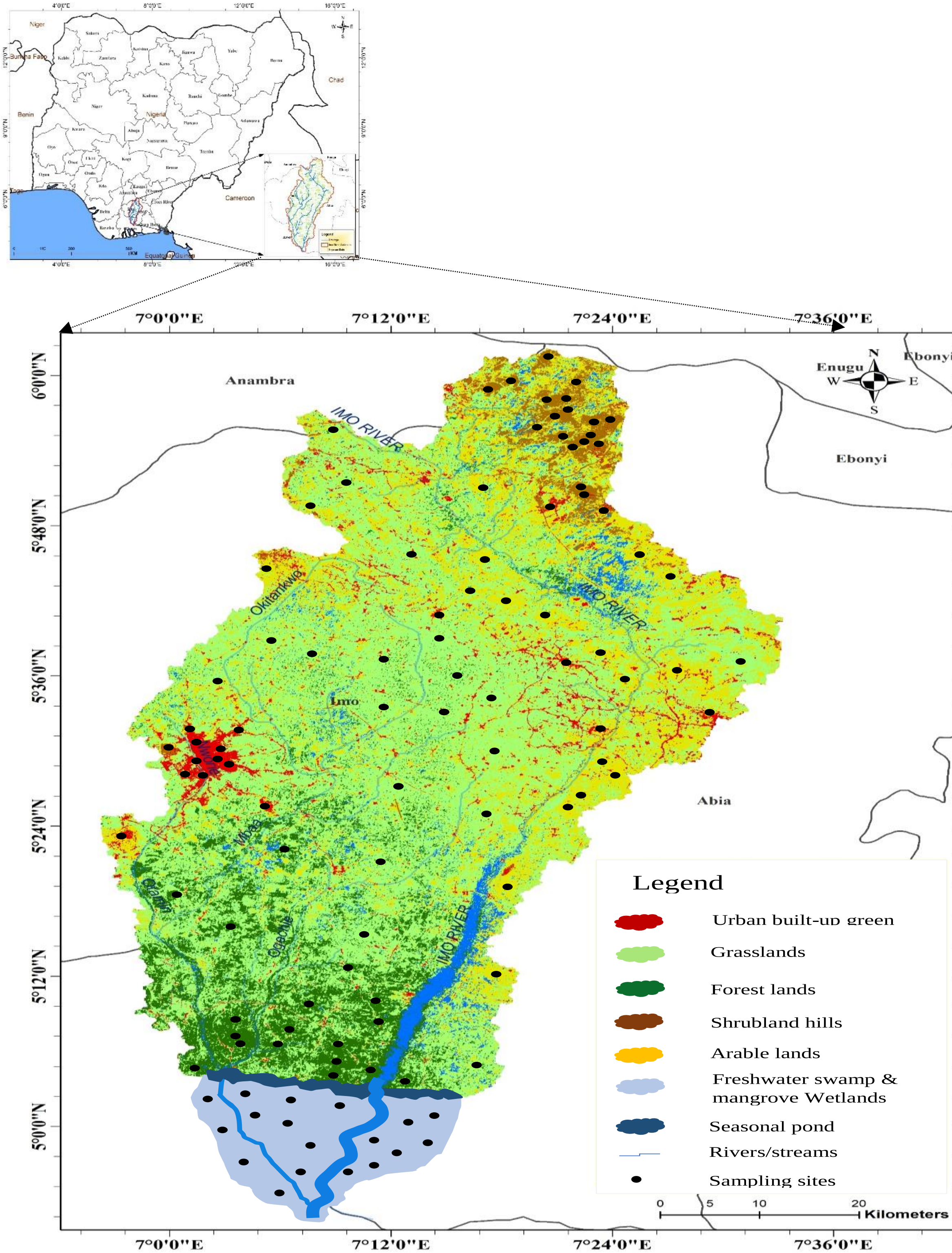

Figure 1. Study area showing land use types, sampling sites, and Imo river watershed and catchment. Arable land (AL), forest land (FL), grassland (GL), shrubland hills (SL), urban built-up green (UL), and the freshwater swamp and mangrove wetland (WL).

The main land uses of the watershed include arable land, forest land, grassland, shrubland on the hills, urban built-up green land, and freshwater swamp and mangrove wetland (Fig. 1). The description of each land use types including the dominant plant species, and area (km^2) for the watershed revealed that the arable land has the largest land area (Table 1). Prior to 1980s, forest land had the largest area, but has been deforested because of high demand for land in agriculture, and other anthropogenic activities caused by rapid population increase in the region. Besides the direct effects, intensive farming and grazing have been indirectly reducing some portions of the forest land which promoting soil erosion (Amangabara 2015). There are very few plantation agricultural areas and more than 85% of the farmers mainly engage in cropping for domestic purposes on individually owned small farmlands. Fallow is not practiced because of population pressure and limited farmlands. Pesticides are not applied, while inorganic fertilizers are marginally used due to inaccessibility and cost, as only few farmers could afford it. Crop residues and manures from the livestock are commonly used as fertilizers. Lands are prepared for cultivation by using local farm tools (hand-held hoes, cutlasses, and rakes) to clear the litters and plow the soil. This tradition helps in abandoning remnants of herbaceous weeds on the soil surface and around the base of the crops to decompose and in turn serves as nutrient replenishment system. The crops are rainfed during the growing seasons (April-November), except during late planting (December-March) when some crops such as *Zea mays* (Maize) and *Talinum fruticosum* (Waterleaf) are cultivated by using manual irrigation. The grasslands are used for animal grazing under open pasture practice belonging to the communities. During the crops growing season, the ruminants are restricted from free browsing.

Table 1

Attributes of the land uses: Arable land (AL), forest land (FL), grassland (GL), shrubland hills (SL), urban built-up green (UL), and the freshwater swamp and mangrove wetland (WL).

Land use	Area (km^2)	altitudinal range (m)	Slope range (^{0})	Main soil type	Communities description and major human activities	Dominant plants species
AL	998.8	396-414	1.5-12	Brown sandy-loam	Cultivation areas with mainly food crops and few cash crops.	*Arachis hypogaea, Manihot esculenta, Dioscorea alata, colocasia, Zea mays, Talinum fruticosum, Abelmoschus esculentus.*
FL	482.1	305-367	3-13	dark-brownish, fine sandy-loam	Thick and broad-leave forest areas with tall trees and large canopies. Logging for timber, firewood, charcoal and oil palm harvest.	*Leucaena leucocephala, Gliricidia sepium, Pentaclethra macrophyllaBenth, Elaeis guineensis, Milicia excelsa, Populas deltoides, Diospyros spp, Nyssa sylvatica, Pterocarpus soyauxii, Entandrophragma cylindricum, Chlorophora excelsa.*
GL	2362	411-583	2-14	Brown coarse sandy-loam	Areas with herbacoius plants species and less than 10% short trees and shrubs cover. Grazing of goats, sheep and cows.	*Andropogon gayanus, Brachiaria decumbens, Cenchrus ciliaris, Cynodon dactylon, Pennisetum pedicellatum, Panicum maximum, Panicum purpureum.*
SL	132.3	578-936	4-27	Red-brownish sandy-loam	A form of transhumance by local communities at rainy season peak. And coal mining. Light farming and hunting	*Lovoa trichilioides Harms, Combretum aculeatum, Dichrostachy cinerea, Grewia mollia, Pliostigma thonaigii, Chromolaena odorata, Vernonia amygdalina.*
UL	182.7	327-359	2-12	Brown coarse sandy-loam	Residential plots with recreational parks, and green gardens. Fruits and nut gathering.	*Dacryodes edulis, Anacardium occidentale, Cocos nucifera, Citrus sinensis, Citrus aurantifolia, Mangifera indica, Psidium guajava, Citrus reticulata, Gmelina arborea.*
WL	163.9	19- 72	1-11	Gleyic-brownish, Clay-loam	Floodplain, freshwater swamps and mangrove-marshes. Extractions of crude oil, palm wine and raffia palms	*Rhizophora racemosa, Avicennia germinans, Rhizophora Mangle, laguncularia racemosas, Nypa fruticans, Paspalum vaginatum, Sparganium eurycarpum, Osmunda cinnamomea, Najas spp.*

Data collection and analyses

Sampling design, sample collection, and analyses

Before sampling in 2016, the land use history was investigated by adopting a field interview with the old village members and the community heads. Historically, the watershed was predominated by mainly forests, and shrubs on the hills, while some swamp and marsh areas were in the south. Six land use types: arable land (AL), forest land (FL), grassland (GL), shrubland hills (SL), urban built-up green (UL), and freshwater swamp and mangrove wetland (WL) under upper, middle and lower slope positions were identified using NigSat image and USGS Landsat image which were classified in ArcGIS 10.1 following FAO landuse-cover classification system.

One hundred and eight composite soil samples [six treatments (land use types), three slope positions, two soil depth layers (0-30cm, 30-60cm), three replicates] were collected for laboratory analyses using a 7.0 cm-diameter soil sampler/auger. Before samples collection, the sampling sites were identified using a hand-held GPS (Garmin GPSMAP 64ST, USA). The soil samples in each land use were acquired to measure the soil physiochemical properties including C and N stocks following IPCC guidelines (IPCC 2006). Soil bulk density samples were derived from the same depth intervals as other soil samples by digging another six soil profiles depth of 60 cm for each treatment/replicate via the core method (Blake and Hartge, 1986). The core samples were collected from the depth intervals using 100 cm^3 volume stainless steel tubes of 5cm by 5.1cm diameter and height respectively. Litterfalls, debris, weeds and plant residues were cleared before performing the sampling in each land use. Laboratory analyses were performed at the accredited Central Laboratory of Obafemi Awolowo University in Ile-Ife, Osun state of Nigeria. Prior to the laboratory analyses, the samples were air-dried at room temperature. Roots and other soil debris were removed, and the specimens were gently sieved using 2 mm mesh.

Water holding capacity (WHC) was calculated (Udom and Ogunwole 2015) as:

$$WHC\ (g\ g^{-1}) = (Wm - Dm) / Dm \qquad (1)$$

where WHC = water holding capacity ($g\ g^{-1}$), Wm = mass of wet soil at 0 kpa (g), and Dm= mass of oven-dry soil (g).

Soil bulk density (BD) was calculated as the ratio of dry soil weight and the volume of the soil (Blake and Hartge (1986). Total soil porosity (Pt) was obtained from known BD and soil particle density (2.65 g cm^{-3}) (Qi et al. 2015). The concentration of soil organic carbon (SOC) was determined according to the Walkley and Black method (Schnitzer 1982), while the soil total nitrogen (STN) concentration was determined using the Kjeldahi method (Bremner and Mulvaney, 1982). Soil pH was estimated by acidometer. Soil bulk density (BD) for each depth interval was used to compute the SOC and STN stocks (Mg ha^{-1}) by applying the formula by Ellert and Bettany (1995):

$$\text{SOC stock (SOCs)} = \text{SOCc} \times \text{BD} \times \text{SD} \times 10000 m^2 ha^{-1} \times 0.001 \text{ Mg kg}^{-1} \quad (2)$$

$$\text{STN Stock (STNs)} = \text{STNc} \times \text{BD} \times \text{SD} \times 10000 m^2 ha^{-1} \times 0.001 \text{ Mg kg}^{-1} \quad (3)$$

where SOCs (or STNs) = Soil Organic Carbon or Total Nitrogen Stock (Mg ha^{-1}),

SOCc (or STNc) = Soil Organic Carbon or Total Nitrogen Concentration (kg Mg^{-1}).

BD = soil bulk density (g cm^{-3}), SD = soil depth (thickness) layer (m).

Measurement of in-situ environmental variables at the sampling sites

The vegetation cover (%) and the dominant plant species in the different land use were

observed and recorded according to Braun–Blanquet method (Diwediga et al. 2015). In addition to the use of GPS, the slope and altitude were extracted from SRTM Digital Elevation Model at 30 m resolution which was used for validation purpose.

Table 2

Soil organic carbon and soil total nitrogen concentrations in relation to land use types, soil depth, and various slope positions of the study area (Mean ± SE)

Soil parameters	Land use	Soil depth (cm)	Slope position Upper	Middle	Lower	Overall
SOC (g kg^{-1})	AL	0 - 30	5.38±0.02	6.49±0.19	9.25±0.11	7.04±0.17d
		30 -60	5.13±0.01	6.27±0.12	9.03±0.13	6.81±0.05d
		Overall	5.26±0.02b	6.38±0.14b	9.14±0.08a	
	FL	0 - 30	14.12±1.86	15.23±1.11	18.11±2.17	15.82±1.95a
		30 -60	11.31±1.23	13.62±2.08	16.05±1.44	13.66±1.81b
		Overall	12.72±2.00c	14.43±1.72b	17.08±2.21a	
	GL	0 - 30	8.10±0.62	10.12±1.19	13.01±1.05	10.41±1.12b
		30 -60	7.69±0.55	8.18±0.61	11.34±0.83	9.07±0.57c
		Overall	7.90±0.81c	9.15±0.92b	12.18±1.90a	
	SL	0 - 30	12.2±1.16	7.70±0.74	8.15±0.35	9.35±0.93c
		30 -60	11.61±1.23	6.30±0.85	7.08±0.68	8.33±0.60c
		Overall	11.91±1.45a	7.00±0.77b	7.62±0.94b	
	UL	0 - 30	6.12±0.68	6.01±0.43	9.21±0.57	7.11±0.31d
		30 -60	6.31±0.20	5.91±0.49	8.4±0.51	6.87±0.74d
		Overall	6.22±0.52b	5.96±0.04b	8.81±0.93a	
	WL	0 - 30	9.27±1.01	8.45±0.83	12.55±2.46	10.09±1.35b
		30 -60	11.29±2.44	11.73±2.09	14.27±3.11	12.43±2.67b
		Overall	10.28±2.08b	10.09±1.71b	13.41±2.02a	
STN (g kg^{-1})	AL	0 - 30	0.51±0.04	0.70±0.05	0.86±0.11	0.69±0.07c
		30 -60	0.35±0.03	0.44±0.01	0.56±0.06	0.45±0.03c
		Overall	0.43±0.01c	0.57±0.07b	0.71±0.06a	
	FL	0 - 30	1.61±0.24	2.31±0.28	3.02±0.24	2.31±0.25a
		30 -60	1.07±0.12	2.22±0.23	2.50±0.29	1.93±0.10ab
		Overall	1.34±0.23b	2.27±0.21a	2.76±0.27a	
	GL	0 - 30	1.01±0.18	1.04±0.09	2.03±0.11	1.36±0.12b
		30 -60	0.92±0.03	1.10±0.16	1.25±0.14	1.09±0.18b
		Overall	0.97±0.17b	1.07±0.10ab	1.64±0.19a	
	SL	0 - 30	1.03±0.05	1.06±0.16	1.24±0.21	1.11±0.15b
		30 -60	0.42±0.14	1.00±0.17	1.16±0.09	0.86±0.08c
		Overall	0.73±0.10b	1.03±0.19a	1.20±0.10a	
	UL	0 - 30	0.36±0.05	0.79±0.21	1.07±0.14	0.74±0.11c
		30 -60	0.41±0.08	0.62±0.09	0.86±0.19	0.63±0.17c
		Overall	0.39±0.01c	0.71±0.11b	0.97±0.06a	
	WL	0 - 30	0.92±0.07	1.08±0.14	1.36±0.17	1.12±0.23b
		30 -60	0.85±0.13	1.19±0.20	1.71±0.34	1.25±0.25b
		Overall	0.89±0.09c	1.14±0.31b	1.54±0.33a	

Note: Overall means followed by the same letter (s) across columns and rows are not significantly different ($P < 0.05$) in terms of slope position, land use types, and soil depth. For the land use types abbreviations: AL = arable land, FL = forest land, GL = grassland, SL = shrubland hills, UL = urban built-up green, WL = freshwater swamp and mangrove wetland.

Statistical analysis

Soil physiochemical variables under the different land use, slope positions, and depths were compared using multiple comparisons of one-way ANOVA (Duncan's method) (Negasa et al. 2017; Daniel et al. 2017; Lian et al. 2013). Differences between means of treatments were considered significant ($P < 0.05$ and $P < 0.001$) using the Tukey's HSD test. A repeated-measures ANOVA was also used to test the joint effects of land use types, soil depth, and slope position on SOC, STN, BD, pH, and WHC. The relationships between soil chemical and physical properties were tested using pairwise correlation adjusted to Bonferroni significance level at 0.05. The IBM SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses.

3.2. Results

SOC and STN concentrations in different land use types, slope position and soil depth

In overall, the mean concentrations of SOC and STN were statistically significant under different slope positions and soil depth across the land use types (Table 2). The concentrations of both SOC and STN in the land use followed the same order of FL > WL > GL > SL > UL > AL. The SOC concentration (g kg^{-1}) was higher in soils under FL, while lower concentrations were found in AL and UL land use types. The SOC concentration in FL recorded more than 90% higher than the concentrations in AL and UL, and more than 50% higher than concentrations under GL and SL in both surface and sub-surface depths. In respect to the slope positions, higher SOC concentration was found under the lower slope in all land use except under SL. Topsoil layer indicated the highest SOC concentration in almost all the land use. Similarly, FL had about three times STN concentration relative to the contents available in AL and UL (Table 2). The lower slope also showed high mean concentration of STN. In terms of the vertical distribution, the topsoil layer recorded higher STN content when compared with a sub-surface layer of the soil.

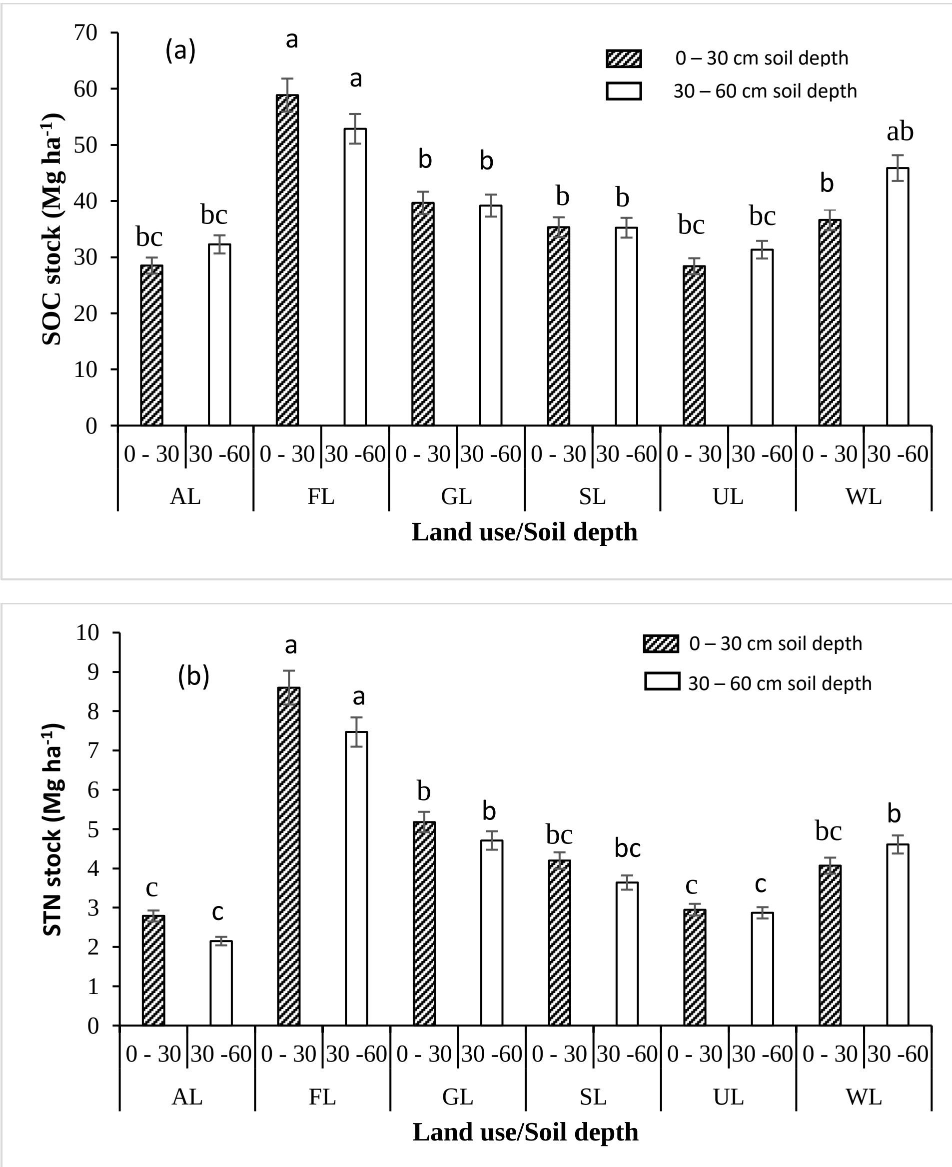

Figure 2. (a) Soil organic carbon stock and (b) Soil total nitrogen stock in relation to different land use types and two levels of soil depths. Columns followed by the same letters for each bar size were not significantly different at $P < 0.05$. For the land use types abbreviations: AL = arable land, FL = forest land, GL = grassland, SL = shrubland hills, UL = urban built-up green, WL = freshwater swamp and mangrove wetland.

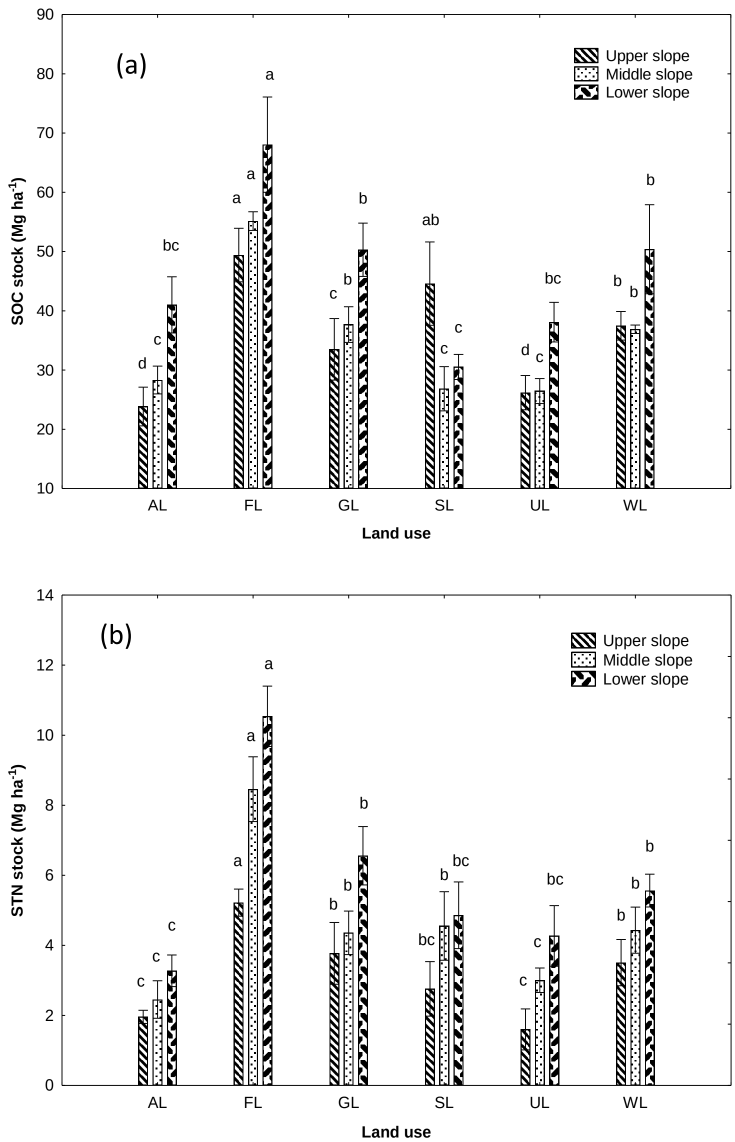

Figure 3. (a) Soil organic carbon stock and (b) soil total nitrogen stock in relation to the different slope positions under various land use types. The error bars represent the standard error of the mean (n = 3). Different letters (a, b, c, and d) indicate significant differences among the various slope positions at $P < 0.05$. For the land use types abbreviations: AL = arable land, FL = forest land, GL = grassland, SL = shrubland hills, UL = urban built-up green, WL = freshwater swamp and mangrove wetland.

SOC and STN stocks in various slop positions, soil depth and under different land uses

Considering the soil depth, FL recorded a significantly high mean stock for SOC and STN, while AL had the lowest when compared with other land use (Figure 2). Topo-sequentially, high mean

SOC stock was found in all slopes under FL with the largest in lower slope (68.05 mg ha−1) (Figure 3(a)). The mean SOC stock was in the order of FL > WL > GL > SL > AL > UL. Lower slope soil had the highest SOC stock across land use except under SL where upper slope had the highest. The mean STN stock value in FL was more than twice higher than the stock found under AL (Figure 3(b)). Upper slope recorded the least stock, while lower slope had the largest in all the land use. The STN stock could be rated as FL > WL > GL > SL > UL > AL in the study.

Table 3

Soil bulk density in relation to slope position, soil depth under the different land use types of the study area (Mean ± SE)

Parameter	Land use	Depth (cm)	Slope position			
			Upper	Middle	Lower	Overall
Bulk density	AL	0 - 30	1.47±0.04	1.45±0.09	1.49±0.10	1.47±0.1a
		30 -60	1.35±0.04	1.39±0.03	1.45±0.05	1.40±0.7a
		0verall	1.41±0.05a	1.42±0.07a	1.47±0.08a	
	FL	0 - 30	1.21±0.03	1.29±0.06	1.22±0.07	1.24±0.6c
		30 -60	1.26±0.08	1.33±0.04	1.28±0.01	1.29±0.6c
			1.24±0.03c	1.31±0.05b	1.25±0.02c	
	GL	0 - 30	1.42±0.08	1.41±0.09	1.46±0.06	1.43±0.3a
		30 -60	1.26±0.08	1.27±0.03	1.28±0.07	1.27±0.2c
			1.34±0.02b	1.34±0.08b	1.37±0.05b	
	SL	0 - 30	1.18±0.07	1.25±0.09	1.35±0.03	1.26±0.5c
		30 -60	1.34±0.03	1.47±0.08	1.42±0.01	1.41±0.6a
			1.26±0.07c	1.36±0.03b	1.39±0.05b	
	UL	0 - 30	1.41±0.09a	1.44±0.06a	1.47±0.07a	1.44±0.4a
		30 -60	1.35±0.05	1.23±0.05	1.41±0.02	1.33±0.1b
			1.38±0.02b	1.34±0.07b	1.44±0.05a	
	WL	0 - 30	1.21±0.01	1.20±0.09	1.25±0.05	1.22±0.2c
		30 -60	1.20±0.03	1.22±0.02	1.27±0.08	1.23±0.3c
			1.21±0.04c	1.21±0.02c	1.26±0.07c	

Note: Overall means followed by the same letter (s) across columns and rows are not significantly different ($P < 0.05$) in terms of slope position, land use types, and soil depth. Upper slope (≥ 11°), middle slope (5-10°), lower slope (≤ 4°); n = 6 number of soil samples in each slope position. For the land use types abbreviations: AL = arable land, FL = forest land, GL = grassland, SL = shrubland hills, UL = urban built-up green, WL = freshwater swamp and mangrove wetland.

Soil bulk density

In overall, arable land (AL) had the highest mean value of 1.47 g cm^{-3} and 1.40 g cm^{-3} BD in both the topsoil and subsurface soil depths respectively (Table 3). GL and UL followed AL in the volume of BD under the different land use types. FL and WL recorded the lowest mean values of soil BD. The relationships between BD and soil depth varied based on land use type. For example, BD was higher in the top soil layer in AL, UL, and GL, whereas the subsoil layer recorded higher under FL, WL and SL. In terms of the topo-sequence, the highest mean values of the bulk densities were found in the lower slope relative to other slope categories.

Table 4

Repeated measure ANOVA for soil properties as jointly influenced by all land use types, soil depth and slope position

Parameters	Df	BD	Pt	SOC	STN	pH	WHC
				MS			
LU	5	0.072**	13.641	1.539**	0.954**	0.018*	1.556*
SD	1	0.011*	9.785	0.342**	0.062**	0.112	1.109*
SP	2	0.035	11.217	0.085**	0.300*	0.737	0.044
LU x SD	5	1.109	4.005	0.009**	0.027**	0.001	0.252
LU x SP	10	0.052	1.338	1.130*	0.601*	0.014	2.685
SD x SP	2	0.008	0.911	0.027	0.015	0.026	0.012
LU x SD x SP	10	0.020	5.704	2.661*	0.003	0.040	0.001

Note: LU = Land Use, SD = Soil Depth, SP = Slope Position, BD = Soil Bulk Density, P_t = Soil Porosity (%), SOC = Soil Organic Carbon (%), STN = Soil Total Nitrogen (%), WHC = Soil Water-Holding Capacity (g g^{-1})), Df = Degree of Freedom. MS = Means.

* $P < 0.05$

** $P < 0.001$

Inter-relationships among soil properties and environmental variables in different land use types

BD was significantly affected by land use ($P < 0.001$) and soil depth ($P < 0.05$) but not with a slope (Table 4). SOC (%) was significantly affected by land use ($P < 0.001$), soil depth ($P < 0.001$), slope position ($P < 0.001$), and the combination of land use and soil depth ($P < 0.001$). On the other hand, the integrated effects of land use and slope position (LU × SP) and the combination of the three parameters (LU × SD × SP) significantly affected the SOC content ($P < 0.05$). Similarly, STN was significantly affected by land use ($P < 0.001$), soil depth ($P < 0.001$), and slope position ($P < 0.05$) (Table 4). The combination of land use and soil depth (LU × SD, $P < 0.001$) and the combination of land use and slope position (LU × SP, $P < 0.05$) showed significant interactive effects on STN content. WHC and pH were significantly affected by land use ($P < 0.05$).

Furthermore, significantly strong correlations ($P < 0.05$) were observed between SOC and STN at both (30 cm and 60 cm) soil depths (Table 5). The soil porosity was also significantly correlated with SOC and WHC, while pH and C:N were negatively correlated with SOC and STN.

3.3. Discussion

SOC and STN concentrations and stocks in different land uses, slope position and soil depth

Anthropogenic disturbances proved to be important factor in both vertical and horizontal distribution of SOC and STN concentrations and stocks, because natural land use types showed elevated contents when compared with agricultural or urban fields in this study. For example, FL had more than 50% content of SOC relative to the value obtained under AL, UL and GL. This result was consistent with other recent studies within and outside same agroecological region with this study (Addis et al. 2016; Diwediga et al. 2017; Diwediga et al. 2015; Jaiyeoba, 1995; Liu et al. 2016; Ma et al. 2015; Rezapour and Alipour 2016; Udom and Ogunwole, 2015).

Table 5.

Correlation matrix between soil chemical and physical properties under different depths (0-30 and 30-60cm)

	SOC30	SOC60	STN30	STN60	C:N30	C:N60	pH30	pH60	WHC30	WHC60	Pt30	Pt60
SOC30	1.00											
SOC60	0.86*	1.00										
STN30	0.61*	0.52*	1.00									
STN60	0.74*	0.65*	0.58*	1.00								
C:N30	-0.35	-0.20	-0.28	-0.15	1.00							
C:N60	-0.12	0.00	0.04	0.10	-0.11	1.00						
pH30	-0.05	-0.16	0.03	0.00	0.05	-0.08	1.00					
pH60	0.08	-0.23	-0.19	0.21	0.02	0.00	0.51	1.00				
WHC30	0.35	0.42	0.60*	0.53	0.04	-0.01	0.09	0.01	1.00			
WHC60	0.48	0.64	0.57	0.40*	0.00	-0.03	0.00	0.12	0.39*	1.00		
Pt30	0.23*	0.19	-0.24	0.08	0.14	0.00	0.07	0.05	-0.46*	0.32*	1.00	
Pt60	0.07	0.31	-0.38	-0.26	0.03	0.00	0.11	0.10	-0.21*	0.20*	0.28	1.00

Note: Correlation coefficients are indicated with asterisk (*) at 0.05 significant level. For abbreviated acronyms, refer to Table 4.

Higher SOC and STN concentrations in FL might be attributed to higher residues decomposition from surface litter input which increased SOC. And increased SOC content has been strongly correlated with high STN content (Diwediga et al. 2017). On the other hand, low SOC and STN contents and stocks in AL, UL, and GL might be explained by unfavorable soil conditions due to the use of plants residues as livestock fodder and fuel, and unsustainable management practices which trigger soil erosion (Addis et al. 2016; Li et al. 2017; Negasa et al. 2017; Udom and Ogunwole, 2015). Cultivation and grazing led to frequent harvesting and uprooting of the plants which consequently removed nutrients from the soil (Haileslassie et al. 2005) and exposed the available OM to soil moisture aeration and decomposing agents. This practice promoted rapid degradation and mineralization of the available OM thus, lowering the SOC and STN concentrations and stocks. Though grazing can increase SOC if managed properly (Xu et al. 2016). Vegetation types and root systems under different land uses have also been reported as a primary factor influencing the contents and stocks of SOC and STN (Li et al. 2017; Wang et al. 2015). For instance, leguminous plant species (such as *Leucaena leucocephala, Gliricidia sepium, Pentaclethra macrophylla B.*), and plant species with broad leaves, large canopies and extensive fine root systems dominated the FL contributing to high SOC and STN compared with other land uses in the study area (Table 1). Besides FL, WL soil have been reported for significant amount of STN and as a large C-reservoir relative to either urban or agricultural lands (Bai et al. 2014; Li et al. 2001; Lugo and Brown 1993; Mathews et al. 2005). In contrast, Ogunkunle and Eghaghara (1992) in southern Nigeria reported higher soil nutrients including total nitrogen and SOM under arable land than either the secondary forest or cocoa plantation. This dissimilarity in results might be due to changes in agricultural practices, climate and vegetation types since there has been long-term gap between the studies.

Landscape position revealed significant influence on SOC and STN concentrations and stocks in the study area. Higher C and N contents were generally recorded in lower slope relative to other slope categories. This could be related to soil erosion and other soil denudational processes which removes nutrients from upper to lower slopes. As human and animal disturbances intensified under AL, GL, and UL especially, the vegetation covers were cleared while the soil aggregates were weakened leading to upward-downward flow mechanism. Therefore, the labile organic C contents accumulate in the lower slope thus, increased the SOC and STN contents. In the same west African region, Diwediga et al. (2017), concurred that lower topography had higher SOC and STN contents relative to other slope categories. In contrary, other authors observed higher SOC and STN in the middle slope (Li et al. 2017) and the upper slope (Li et al. 2015). The discrepancy of the result with this present study could be linked to several influencing factors such as micro-topographical climate, lithological or geomorphological characteristics, types of vegetation cover on the slope, tillage and intensity of different human activities. For example, in this study, upper slope had higher concentration of SOC under shrubland hills (SL), probably because the hills are dominated by granitic materials especially biotites and kaolinite (clay) which are highly resistance to erosion and surface runoff (Pleessel 1982).

Soil depth revealed significant effect on SOC and STN contents with higher concentrations found in the topsoil (0-30cm) layer. This result was consistent with several studies in this concept (Wang et al. 2016). Elevated litterfall and residues input might be the reason for increase SOC and STN contents and stocks in the topsoil. Dense concentrations of the root system at the topsoil have been revealed as another reason for high SOC and STN contents at the A-horizon. Though this reason is not applicable across land uses in this study because FL has roots beyond the 30cm depth. Contrary to other land uses, WL soil showed higher SOC and STN in the sub-surface soil depth.

This might be attributed to moisture content, plant types, and human activities (such as mud-fishing, local gin distillation, palm-wine and crude-oil extractions) which reduced soil C and N from top soil in the wetland. However, some other researchers have reported lower soil C and N in the surface soil relative to the subsoil (Gelaw et al. 2014; Wiesmeier et al. 2012). The disparity with this study is likely related to increase in the root litter and downward SOC transport by bioturbation or soil water percolation (Wang et al. 2016), or due to high ground water table which leads to accumulation of OM in the subsoil (Wiesmeier et al. 2012).

Soil bulk density

The overall mean BD in AL was higher than that found in other land use types. This could be attributed to the traditional agricultural practices such as tillage, litter removal and intensive cultivation which in turn decreased SOC (by rapid mineralization of SOM) and consequently elevated the BD. Several studies have confirmed the effects of tillage on BD and SOC (Addis et al. 2016; Negasa et al. 2017). A recent study in the northern highlands of Iran revealed the compaction of surface soil layer due to intensive cropping which apparently led to the increase in BD (Emadi et al. 2008). GL also recorded higher BD because of ruminant activities which caused severe soil compaction in the study area. This finding conformed to previous works on the role of livestock and man on the increase of BD and degradation of arable and urban soils in Beijing (Liu et al. 2018). FL and WL recorded lowest BD, and this might be connected to the natural characteristics of these ecosystems when compared with disturbed arable and grasslands. A contrasting result was reported in a loess hilly-gully catchment of China, where cropland had the lowest BD (Liu et al. 2018). The disparity in the results could be attributed to differences in physical geographical features (climate, soil, slope) and agricultural management systems. Natural land use types such as FL and WL soil have higher SOC due to continuous addition of SOM and plant residues which consequently decreased the BD. This result agreed with the studies from the watershed in Ethiopian highland, and Northern China as reported by Addis et al (2016) and Wang et al. (2016) respectively. Another study in southern Nigeria by Udom and Ogunwole (2015) also found lower BD in forested areas, but high in cultivated soil areas. With respect to soil depth, higher BD was found in the top soils under AL, UL, and GL relative to FL, SL and WL soils. This could be explained by lower organic matter content in the surface soil in agricultural areas caused by anthropogenic disturbances (Gelaw et al. 2015). This result was inconsistent with some authors' study which reported increase in BD with depth (Qi et al. 2018). Differences in soil compositions and prevailing farming techniques might be reasons for this dissimilarity in results.

Inter-relationships among soil properties and environmental variables

The soil C:N ratio and pH were negatively correlated with SOC and STN contents. This might be due to increase in deforestation. It has been reported that removal of vegetation cover leads to a drop in the values of the soil productivity index which subsequently decreases soil organic matter but elevated C:N ratio (Six et al. 2000). Other authors have reported that C:N ratio decreases with an increase in SOC and STN due to rapid decomposition (Narayan and Anshumali, 2016). WHC was significantly related with SOC content because higher soil water content can solute more C fractions into soils (Bai et al. 2014). The observed significant positive correlation between soil porosity, SOC, and WHC could be because SOC promotes the binding between the soil particles, leading to the formation of stable soil aggregates, thus, soil porosity increases by absorbing more rainwater inflow and reducing the runoff (Hugar 2017).

3.4. Conclusions

The findings of this study revealed that land use, slope position, and soil depth significantly affected SOC and STN concentrations and stocks as well as BD. AL had higher BD, while FL and WL recorded the lowest: an indication that undisturbed ecosystems have lower BD than the disturbed systems. This might be related to a decrease in SOC due to poor agricultural practices (such as burning, tillage, and litter removal) without conservation methods. SOC and STN concentrations and stocks were remarkably higher under FL relative to AL, GL, and UL, indicating that afforestation, forest, and wetland conservations are appropriate for improving soil quality and increase soil C and N sequestration in the region. The low nutrients under the agriculture and urban land systems call for the urgent need to introduce conservation agriculture which would reduce intensive grazing, over-cropping, and tillage. The study observed high concentrations of carbon and nitrogen in the topsoil under FL and WL. This information will help to guide against the risk of releasing excess CO2 from the topsoil by converting either FL or WL into agricultural lands. Therefore, the study provides the agriculturists, land managers, and other stake-holders with the needed information to improve soil quality, conserve C and N stocks for food security, sustainable environment, and mitigation of climate change.

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CHAPTER FOUR

Plant species composition responses to long-term effects of mulching, traditional cutting and no management of improved upland grassland, Czech Republic

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Abstract

A shortage of available livestock for utilizing grassland biomass in Central Europe is challenging for the management of both semi-natural grasslands and previously intensified (limed, fertilized and reseeded) upland grasslands. An alternative method of grassland management is mulching, in which aboveground biomass is cut, crushed and subsequently spread on the surface. This paper reports on an experiment to compare three different mulching frequencies (one, two and three times per year) with an unmanaged treatment and traditional management of two cuts per year (control) on a previously improved upland meadow. Plant species composition was monitored over 13 years. Traditional management of two cuts with biomass removal was the most suitable method for maintaining plant species richness and diversity, and both were reduced significantly in the once-mulched and especially in the unmanaged treatment. Tall dicotyledonous weeds such as Urtica dioica, Cirsium arvense and Aegopodium podagraria were promoted by the unmanaged treatment and by mulching once a year. Higher frequency of defoliation had positive effects on the spread of short forbs such as *Taraxacum spp.*, *Plantago lanceolata* and *Trifolium repens*. After eight years, there were changes in sward structure in the unmanaged and mulched-once- a- year treatments, with increase in the tall/short species ratio. In conclusion, repeated mulching cannot substitute fully for traditional two-cut management in improved upland meadows without decreasing plant species richness and diversity and changing the sward structure. Although mulching once a year may prevent invasion by shrubs and trees, it also supports the spread of weedy species similar to no management.

Key words: Abandonment, botanical composition, diversity, extensification, functional groups, succession

4. Introduction

Permanent grasslands cover substantial parts of the landscape of Central Europe. In the 1990s, political changes in Central and Eastern Europe connected with economic transformation and restructuring led to a reduction in the market for agricultural products, and this in turn resulted in a collapse of agricultural production. In the Czech Republic, the importance of grasslands for fodder production decreased dramatically due to a rapid reduction in cattle and sheep herds, together with an increase in grassland in upland areas on former arable fields (CSO 2012; Gaisler et al. 2013). This resulted in extensification or abandonment of many areas of previously intensively managed grasslands. At the beginning of the 21st century, it was estimated that 30% of the total grassland area in the Czech Republic consisted of non-utilized meadows and pastures (Hrabě and Müller 2004). In abandoned grasslands, a rapid decrease in the number of vascular plant species, and/or disappearance of rare or endangered plant species has been reported (Krahulec et al. 2001; Mašková et al. 2009; Pavlů et al. 2005; Weiss and Jeltsch 2015). If the cultural landscape in Central Europe is to be maintained, and the land kept in agricultural condition while also ensuring the provision of other ecosystem services, it is important that grasslands should be maintained using suitable and economically acceptable methods of grassland management. The decades of the 1970s and 1980s were the peak period in the Czech Republic for the establishment and state support of improved grasslands. For example, 48,000 ha of meadows and pastures were ploughed and reseeded using productive species and 33,000 ha out of a total of 724,000 ha of grasslands in the Czech Republic were drained in the 1970s (Klesnil et al. 1982). These grasslands were established using highly productive grass-clover seeds mixtures that were species poor and based mainly on the following: *Dactylis glomerata, Festuca pratensis, Lolium perenne, Phleum* *pratense, Trifolium pratense and Trifolium repens.* To avoid undesirable changes in botanical composition of grasslands that are not currently in agricultural use, mulching has been used since the 1990s in the Czech Republic as a low-cost alternative to grazing or conventional mowing (Fiala 2007; Gaisler et al. 2010; Gaisler et al. 2013). The method involves cutting the aboveground biomass without removal; the clippings being crushed into pieces of several cm lengths and then spread on the grassland surface (Doležal et al. 2011; Metsoja et al. 2012). There are likely to be different responses to mulching management between semi-natural and improved grasslands because of differences in nutrient status, botanical composition and management history. There have been several studies dealing with the effects of mulching management (Bakker et al. 2002; Duffková 2008; Kahmen and Poschlod 2008; Kahmen 2002; Mašková et al. 2009; Römermann et al. 2009; Tonn and Briemle 2008); however, few of these were conducted over the long term (Gaisler et al. 2013). In a previous paper (Gaisler et al. 2013), we reported results from a long-term experiment on the different terms and frequency of mulching and its effect on vegetation of an area of upland semi-natural Festuca rubra-dominated, species-rich grassland with low soil nutrient status. Results indicated that mulching performed at least twice a year was able to substitute for cutting management in this type of low-production grassland, without incurring substantial loss of plant species richness and diversity. It was also found that mulching only in September changed the vegetation of the grassland in ways similar to having no management, and therefore, this option was considered suitable only as a means of preventing the establishment of shrubs and trees.

However, there is no information about the long-term effect of this alternative method of management on the vegetation of these formerly improved grasslands. To address this knowledge gap, in 2000 a grassland management experiment was established on a previously improved and managed upland meadow (i.e., sown with a high-production grass-clover seed mixture, limed and fertilized). Analyses performed on this experiment in 2011 revealed that 11 years of different mulching regimes had not caused any substantial changes in soil and herbage nutrient concentrations in comparison with the unmanaged or/and cut treatments (Pavlů et al. 2016). Thus, based on these results, we concluded that mulching management performed two or three times per year could substitute for a conventional cutting regime. However, in the paper of Pavlů et al. (2016) detailed evaluation of vegetation development was not taken into account.

Therefore, the aim of the study reported in this paper was to evaluate the effects of different mulching management regimes on vegetation characteristics of a previously improved upland meadow over a 13-year period. In the present paper, we have focused on the following questions: (a) how do different meadow management methods (mulching, cutting and abandonment) affect long-term successional changes in plant species composition, the main plant functional groups and species richness? and (b) At what point in time can the key changes after different management introduction can be detected and are there any sward characteristics that can be used to provide a simple predictor for them?

4.1. Materials and methods

Study site

The experiment was established in the Jizerské hory Mts. (50°51′N, 15°02′E; 443 m elevation) 10 km north-west of Liberec, in the north of the Czech Republic. The site has a mean annual temperature of 7.2°C and average annual precipitation of 803 mm (Liberec meteorological station). The soil is acid Cambisol overlying orthogneiss. Soil chemical properties in the 0-10 cm soil layer, measured at the beginning of the experiment in 2000, were as follows: pH/KCl 6.3 (pH/H2O 6.8), organic C content 2.7%; and plant-available (Mehlich III extraction; Mehlich 1984) P, K and Mg concentrations 28, 138 and 290 mg/kg respectively. Mean soil chemical properties for individual treatments after 11 years of the applied management are given in Table 1 (for details see Pavlů et al. 2016).

Table 1
Mean soil chemical properties for the different treatments measured in 2011 (Pavlů *et al.*, 2016). Numbers represent mean values for each treatment: two cuts per year (2C), unmanaged (U), mulching once per year (1M), mulching twice per year (2M) and mulching three times per year (3M).

	Characteristic	Treatment				
		2C	U	1M	2M	3M
Soil	pH/KCl	5.87	6.56	6.40	6.12	6.08
	C_{org} mg kg^{-1}	28500	30350	30050	30875	30075
	N_{tot} mg kg^{-1}	2200	2440	2508	2503	2580
	P mg kg^{-1}	17.26	34.14	26.29	25.40	20.08
	K mg kg^{-1}	69.50	157.59	141.72	125.47	100.44
	Ca mg kg^{-1}	2053	2654	2464	2193	2234
	Mg mg kg^{-1}	282.4	388.0	350.5	345.3	371.4
	C:N	13.0	12.5	12.0	12.3	11.7

In 1990, ten years before establishment of the experiment, the site was agriculturally improved by draining, then limed (500 kg CaO/ha), fertilized (100 kg N/ha, 40 kg P/ha, 80 kg K/ha) and reseeded with a grass-clover mixture suitable for high forage production, comprising *D. glomerata, F. pratensis, P. pratense, T. pratense* and *T. repens*. From 1991 to 2000, the meadow was cut twice each year and occasionally grazed by cattle. The plant community of the study area was classified as Arrhenatherion alliance (Chytrý 2007), and before the start of the study in 2000, the dominant vascular plant species were *D. glomerata, F. pratensis, P. pratense, Galium album,* and *Veronica chamaedrys*. There were no fertilizer applications to the meadow for at least five years prior to the start and during the period of the experiment. The average yield of forage was about 5 t/ha of dry matter (DM) per year.

Experimental design

The experiment was carried out in four completely randomized blocks during the period 2000 to 2013. In each block, there were rectangular (10 m × 5 m) treatment plots, one for each of five treatments. The following treatments were applied: unmanaged (U), two cuts per year (as a control) with removal of cut biomass in June and August (2C), mulching performed once per year in July (1M), mulching twice per year in June and August (2M) and mulching three times per year in May, July and September (3M). A tractor-driven mulching machine (Uni Maher UM 19, Gerhard Dücker GmbH & Co. KG) was used for the mulching treatments: plant biomass was crushed into pieces 5–10 cm long, spread on the sward surface and pressed by the roller. Cutting was carried out using a tractor-driven rotary mower and cut biomass (in the 2C treatment) was removed immediately following cutting. The residual sward height after mulching and cutting was approximately 5 cm.

Plant species composition and functional groups

The coverage of all vascular plant species was determined by visual estimation and recorded directly on a percentage scale. To avoid possible plot-edge effects, only the central area of 8 m × 3 m of each permanent plot was used for the observations. Collection of relevés was carried out annually at the end of May before the first mulching application. Nomenclature of vascular plant species follows Kubát et al. (2002). Weed species in grasslands were considered to be those plant species that (a) significantly reduced forage quality, and/or (b) decreased biomass yield (Mikulka et al. 2009). A list of weedy species is presented in Supporting Information Table S1.

In accordance with the mean height of vascular plants as listed in the regional flora (Kubát et al. 2002), all plant species recorded in the experiment were a priori categorized into four main groups: short graminoids, tall graminoids, short forbs and tall forbs. The tall/ short species ratio was also recorded. Species with a mean height of ≥ 0.5 m were considered tall, and those below this threshold were considered as short (see Supporting Information Table S1). Shannon diversity (H index) was calculated from the cover data of all vascular plant species in a particular relevé (Begon et al. 2005).

The C-S-R functional types (Grime et al. 1988) were used for evaluating changes in the sward under each different management. Data for determination of C-S-R strategies for each plot were calculated from means of C, S and R values, weighted using the cover of each vascular plant species present in the individual plot, where the sum of all strategies was 1 (100%) (Hunt et al. 2004). Ellenberg nutrient indicator values (EIV) for nutrients, light and soil moisture for each relevé were calculated as the mean of the indicator values (Ellenberg et al. 1992), weighted according to the cover for each vascular plant species.

Data analyses

Redundancy analysis (RDA) in the CANOCO 5 program (ter Braak and Šmilauer 2012) was used in order to evaluate trends in plant species composition. Cover data in RDA were logarithmically transformed [y = log (y + 1)] before analyses in order to down-weight dominant species (Lepš and Šmilauer 2003). A total of 999 permutations were used in all the performed analyses. Blocks were used as covariables in all analyses. The tested null hypotheses were as follows: A1) the temporal trend in the species composition is independent of the treatment, and A2) there are no directional changes in time in the species composition that are common to all treatments. The data are formed by repeated observations including baseline data. Therefore, the interaction of treatment and year (numeric) were the most important variables. A standard biplot ordination diagram was used to visualize the results of the RDA analysis. A linear mixed-effects model with fixed effects of treatment, time (factor) and their interaction and random effect of replication was used to evaluate the cover of the most abundant vascular plant species, H index and richness, cover of functional groups, tall/short species ratio and C-S-R strategy. A linear mixed-effects model with fixed effect of treatment and random effect of replication was used to test differences of the cover of the most abundant vascular plant species, species diversity and richness, cover of functional groups, tall/short species ratio and C-S-R strategy in the year 2013. Replication was used as covariate with random effect in all performed analyses. Further, to identify significant differences between individual treatments a post-hoc comparison using Tukey's HSD test was applied. There was no transformation as the data met all requirements of ANOVA. All univariate analyses were performed using Statistica 13.1 (Dell Inc 2016).

4.2 Results

Species richness and diversity

The species richness of all vascular plants, number of species with a cover value ≥1% and the Shannon index of diversity (H) were all significantly affected by time, treatment and by time × treatment interaction (Table 2). Before the start of the experiment, the total number of vascular plant species was about 30 per plot (24 m2) in all treatments.

Table 2
Results of linear mixed-effects models for all years (fixed: time, treatment, time×treatment, random: replication) and for 2013 (fixed: treatment, random: replication) for number of plant species, biodiversity, functional groups, CSR strategy, Ellenberg indicator values (EIVs) and dominant plant species.

	Effect							
	Time; *df*=13		Treatment; *df*=4		Time × treatment; *df*=52		Treatment in 2013; *df*=4	
	F-ratio	*p*-value	*F*-	*p*-value	*F*-ratio	*p*-value	*F*-ratio	*p*-value
Number of all plant species	3.20	<0.001	213.5	<0.001	4.00	<0.001	33.24	<0.001
Number of species with cover≥1%	9.56	<0.001	53.92	<0.001	2.36	<0.001	7.70	0.003
Shannon index of diversity H	6.70	<0.001	120.2	<0.001	3.20	<0.001	42.67	<0.001
Cover of short graminoids	4.88	<0.001	20.97	<0.001	1.75	0.003	0.80	0.546
Cover of tall graminoids	28.01	<0.001	10.70	<0.001	3.97	<0.001	9.99	0.001
Cover of short forbs	14.20	<0.001	231.1	<0.001	3.90	<0.001	70.76	<0.001
Cover of tall forbs	31.00	<0.001	95.67	<0.001	4.12	<0.001	45.09	<0.001
Cover ratio (tall / short species)	21.27	<0.001	94.16	<0.001	9.32	<0.001	24.78	<0.001
C - strategy	17.00	<0.001	342.6	<0.001	5.50	<0.001	73.51	<0.001
S - strategy	18.90	<0.001	303.0	<0.001	5.30	<0.001	69.22	<0.001
R - strategy	14.20	<0.001	359.3	<0.001	5.50	<0.001	75.46	<0.001
EIV for nutrients	7.83	<0.001	33.43	<0.001	2.07	<0.001	21.74	<0.001
EIV for light	11.64	<0.001	54.61	<0.001	2.48	<0.001	5.66	0.009
EIV for moisture	30.80	<0.001	99.87	<0.001	4.61	<0.001	26.24	<0.001
Arrhenatherum elatius	10.10	<0.001	64.22	<0.001	4.92	<0.001	9.22	0.001
Dactylis glomerata	13.10	<0.001	80.09	<0.001	3.87	<0.001	13.02	<0.001
Elytrigia repens	18.39	<0.001	71.78	<0.001	1.27	0.127	10.07	0.001
Festuca rubra	5.27	<0.001	90.02	<0.001	1.48	0.029	46.46	<0.001
Holcus lanatus	8.78	<0.001	40.38	<0.001	2.64	<0.001	9.57	0.001
Aegopodium podagraria	10.34	<0.001	54.47	<0.001	3.38	<0.001	18.65	<0.001
Galium album	19.72	<0.001	38.57	<0.001	1.97	<0.001	5.82	0.008
Taraxacum spp.	25.50	<0.001	234.9	<0.001	7.90	<0.001	107.30	<0.001
Trifolium repens	13.44	<0.001	79.14	<0.001	4.13	<0.001	3.20	0.053
Urtica dioica	10.80	<0.001	171.3	<0.001	8.10	<0.001	18.93	<0.001

Note: df = degrees of freedom, *F* = value derived from F statistics in repeated measurements ANOVA, *p* = probability value.

After 13 years of the different management regimes, the mean numbers of vascular plant species differed significantly according to management intensity in the order 2C > 3M, 2M >1M, U. There was a gradual increase in vascular plant species number in the multiple-managed treatments during the course of the experiment, especially in the 2C treatment where biomass was removed immediately after the cut (Figure 1a).

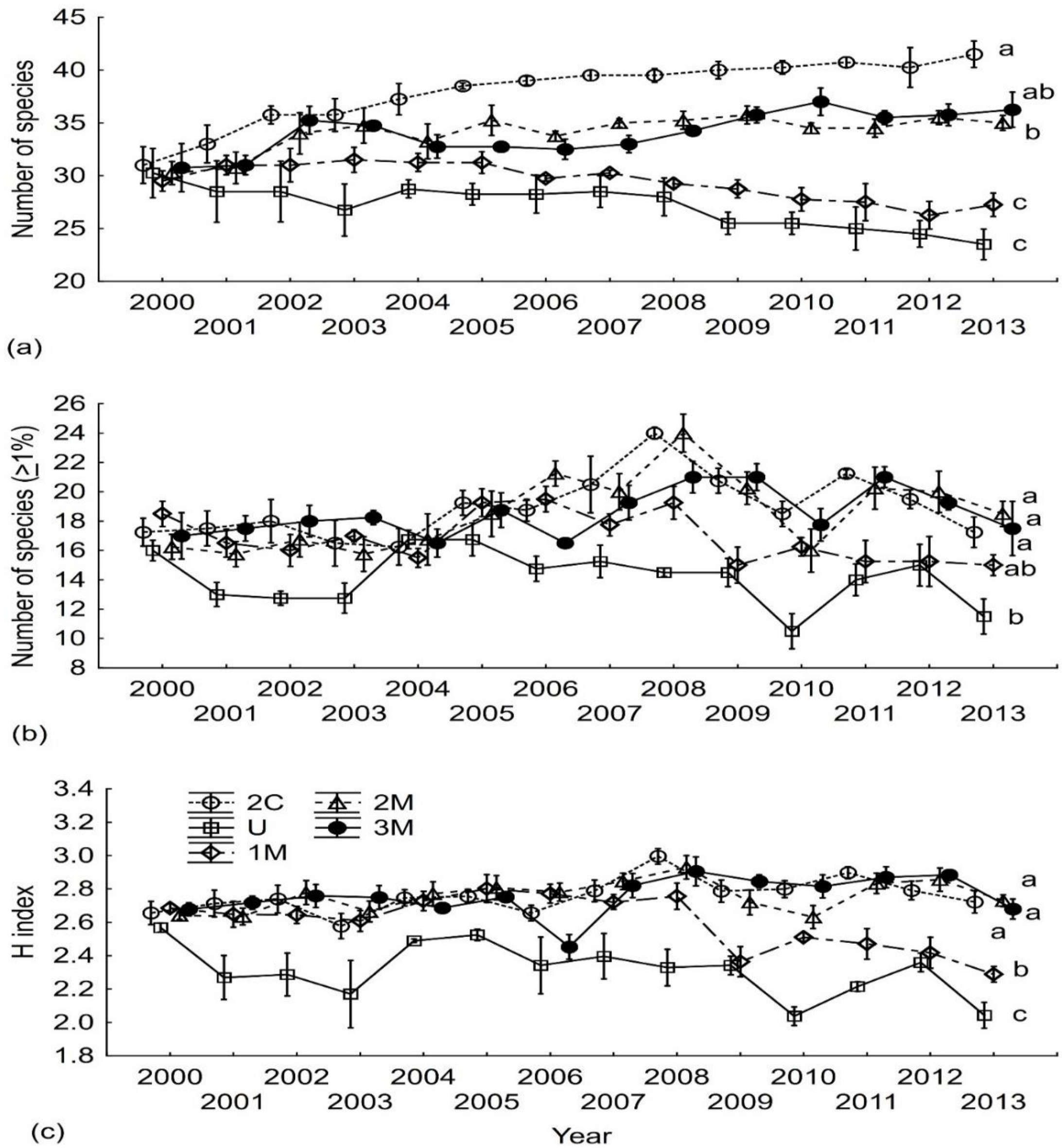

Figure 1. Changes in plant species composition of the tested management treatments between 2000 and 2013. (a) changes in mean number of vascular plant species per 24 m^2, (b) changes in mean number of vascular plant species that contributed ≥ 1% cover, (c) changes in mean Shannon species diversity (H index). Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatments were: unmanaged control (U), two cuts a year with biomass removal in June and August (2C), mulching once a year in July (1M), mulching twice a year in June and August (2M) and mulching three times a year in May, July and September (3M).

Table 3
Results of tested hypotheses to explain differences among the studied management treatments and changes over time independent of treatment, based on Redundancy Analysis. Tested null hypotheses were: A1– the temporal trend in the species composition is independent of the treatment, and A2–there are no directional changes in time in the species composition that are common to all treatments. Treatments were unmanaged control (U), two cuts a year with biomass removal in June and August (2C), mulching once a year in July (1M), mulching twice a year in June and August (2M) and mulching three times a year in May, July and September (3M).

Tested hypotheses	Explanatory variables	Covariables	% expl. var. 1[st] axis, 2[nd] axis (all axes)	*F*-ratio 1st axis (all axes)	*p*-value 1st axis (all axes)
A1	Year ˣ 2C, Year ˣ U, Year ˣ 1M, Year ˣ 2M, Year ˣ 3M	Year, Plot ID, Block	26.69, 38.06 (43.54)	99.4 (52.6)	0.001 (0.001)
A2	Year ˣ 2C, Year ˣ U, Year ˣ 1M, Year ˣ 2M, Year ˣ 3M, Year	Plot ID, Block	29.17, 36.4 (41.39)	112 (48.2)	0.001 (0.001)

Note: ˣ indicate interactions of environmental variables; % expl. var. = species, variability explained by one (all) ordination axis (measure of explanatory power of the explanatory variables); *F*-ratio = *F* statistics for the test of particular analysis; *p*-value = probability value obtained by the Monte Carlo permutation test.

In contrast, there was a gradual decrease in the number of vascular plant species in the U and 1M treatments from 2008 onwards. The number of vascular plant species with ≥1% cover did not relate to treatment in the same way as the total species richness related to treatment (Figure 1b). After 13 years of the experiment, the significantly lowest values in the number of vascular plant species were revealed in the U and 1M treatments. Plant species diversity expressed by the Shannon H index was relatively stable in the 2C, 2M and 3M treatments, whereas it decreased in the U and 1M treatments (Figure 1c), where the lowest value was revealed in the final experimental year.

Plant species composition

There were significant differences among the study treatments and also some remarkable changes over time independent of treatment were detected by the RDA analysis (Table 3). The treatments that had similar plant species composition according to the first ordination axis were sorted into two main groups: U and 1M treatments, and all of the multiple-managed treatments (2C, 2M, 3M)

(Figure 2). Although there was a reduction in the cover of the originally sown species *F. pratensis* in all treatments, its presence was supported by management with multiple mulching (2M, 3M) (Figure 2). On the other hand, the cover of the weed grass E. repens decreased especially in the multiple-managed treatments, although it increased in the U treatment, where it attained its highest cover value in the final year of the experiment (Figure 3c).

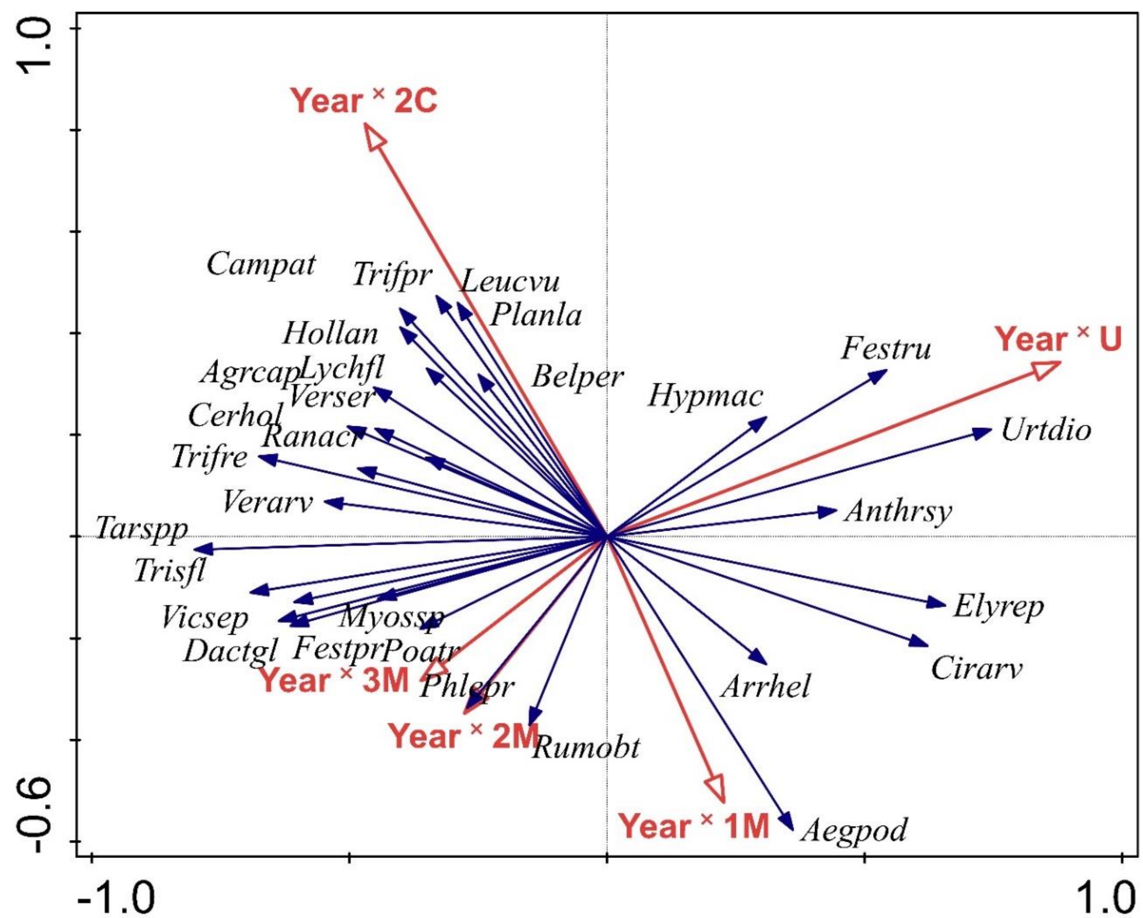

Figure 2. Ordination diagrams showing the results of redundancy analysis of plant species composition data (see Table 3 for details). Treatment abbreviations (U, 2C, 1M, 2M and 3M) are explained in Figure 1; × indicate interactions of environmental variables; Species abbreviations: *Aegpod* = *Aegopodium podagraria*, *Agrcap* = *Agrostis capillaris*, *Antsyl* = *Anthriscus sylvestris*, *Arrela* = *Arrhenatherum elatius*, *Belper* = *Bellis perennis*, *Campat* = *Campanula patula*, *Cerhol* = *Cerastium holosteoides*, *Cirarv* = *Cirsium arvense*, *Dactgl* = *Dactylis glomerata*, *Elyrep* = *Elytrigia repens*, *Fespra* = *Festuca pratensis*, *Fesrub* = *Festuca rubra*, *Hollan* = *Holcus lanatus*, *Hypmac* = *Hypericum maculatum*, *Leucvu* = *Leucanthemum vulgare*, *Lychfl* = *Lychnis flos-cuculi*, *Myospp.* = *Myosotis* spp., *Phlepr* = *Phleum pratense*, *Plalan* = *Plantago lanceolata*, *Poatr* = *Poa trivialis*, *Ranacr* = *Ranunculus acris*, *Rumobt* = *Rumex obtusifolius*, *Tarspp* = *Taraxacum* spp., *Trifpr* = *Trifolium pratense*, *Trifre* = *Trifolium repens*, *Trisfl* = *Trisetum flavescens*, *Urtdio* = *Urtica dioica*, *Verarv* = *Veronica arvensis*, *Verser* = *Veronica serpyllifolia*, *Vicsep* = *Vicia sepium*.

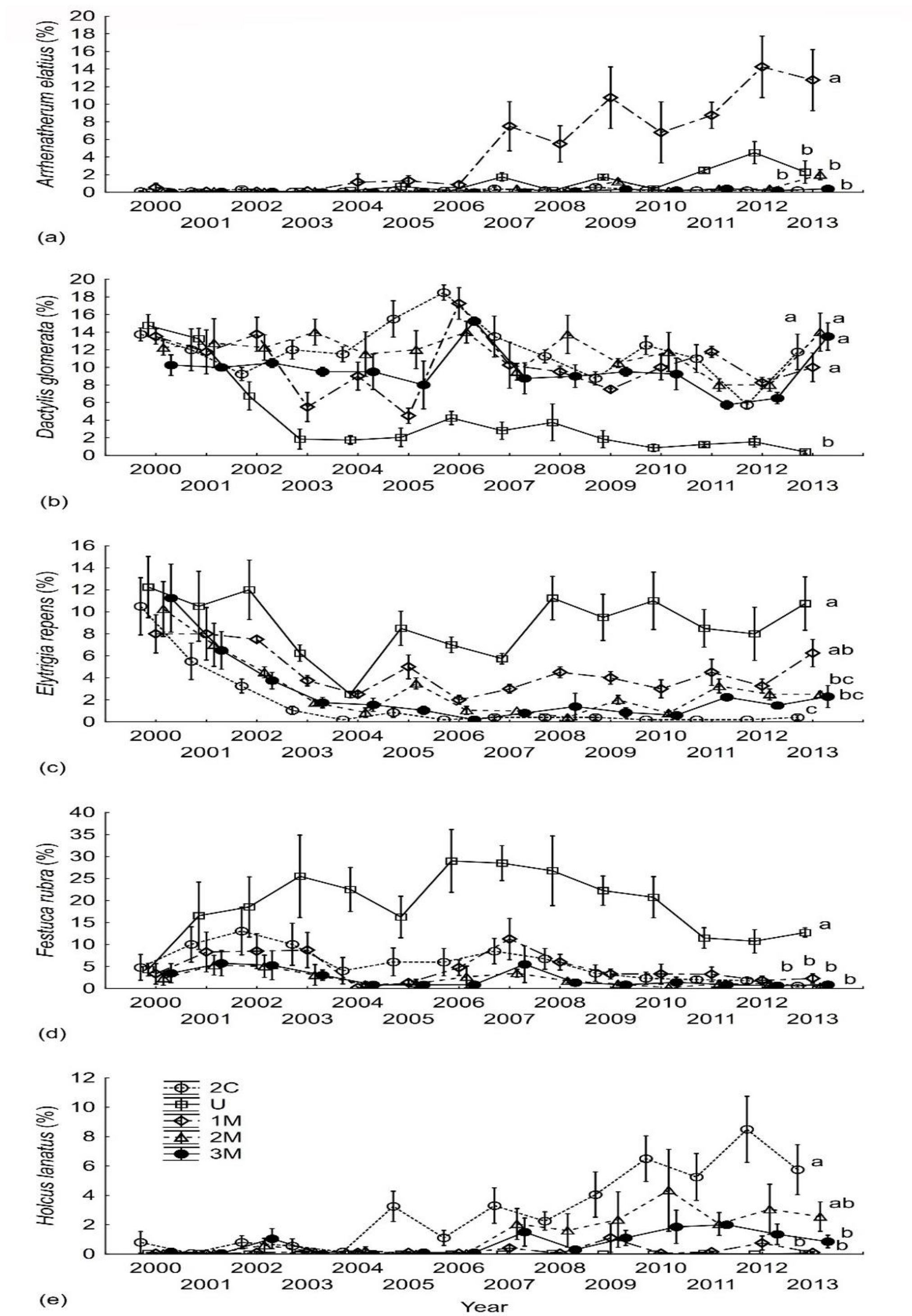

Figure 3. Changes in mean cover values of the dominant grass species – (a) *Arrhenatherum elatius*, (b) *Dactylis glomerata*, (c) *Elytrigia repens*, (d) *Festuca rubra* and (e) *Holcus lanatus* in the investigated treatments between 2000-2013. Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatment abbreviations (U, 2C, 1M, 2M and 3M) are explained in Figure 1.

At the beginning of the experiment, the occurrence of *Arrhenatherum elatius* was negligible in all treatments, but in subsequent years, it increased under mulching in the 1M treatment (with a cover more than 10%). In this treatment, the final cover was the highest after 13 years of the experiment (Figure 3a). Its cover slightly increased on the unmanaged plots, but there were only a few plants recorded in the 2C and 3M treatments. The unmanaged treatment was also favourable for *F. rubra agg*. Its cover increased from 5% in the year 2000 to 13% in the final year the experiment, although in some years it was more than 25% (Figures 2 and 3d). The cover of *Holcus lanatus* increased in the 2C treatment from 2008 onwards (Figures 2 and 3e). The lowest cover of the originally sown tall-grass species *D. glomerata* was in the U treatment; this was already apparent in the third year (Figures 2 and 3b). The multiple managed treatments (2C, 2M, 3M) were favourable for short forbs with creeping growth or prostrate rosettes, such as *T. repens* and *Taraxacum spp*., although the prostrate forb *P. lanceolata* was supported by the 2C treatment only (Figures 2 and 4). The positive effects of multiple-management treatments on the cover of T. repens disappeared from 2008 onwards, though not for *Taraxacum spp.* The successive increase in the cover of tall weedy forbs as *Urtica dioica, C. arvense* and *A. podagraria* was supported by the U and 1M treatments, but these forbs were suppressed in all of the multiple-managed treatments (Figures 2 and 4). The cover of *G. album* was increased in all experimental treatments, and the highest cover was revealed in the 1M treatment (Figure 4b). In the U treatment, woody species occurred 5 years after abandonment. However, they were present only sporadically in one block and therefore not introduced to analyses.

Functional groups, C-S-R strategies and EIV

The cover of short and tall graminoids, as well as short and tall forbs and the tall/short species ratio, were affected significantly by time, treatment and by time × treatment interaction (Table 2).

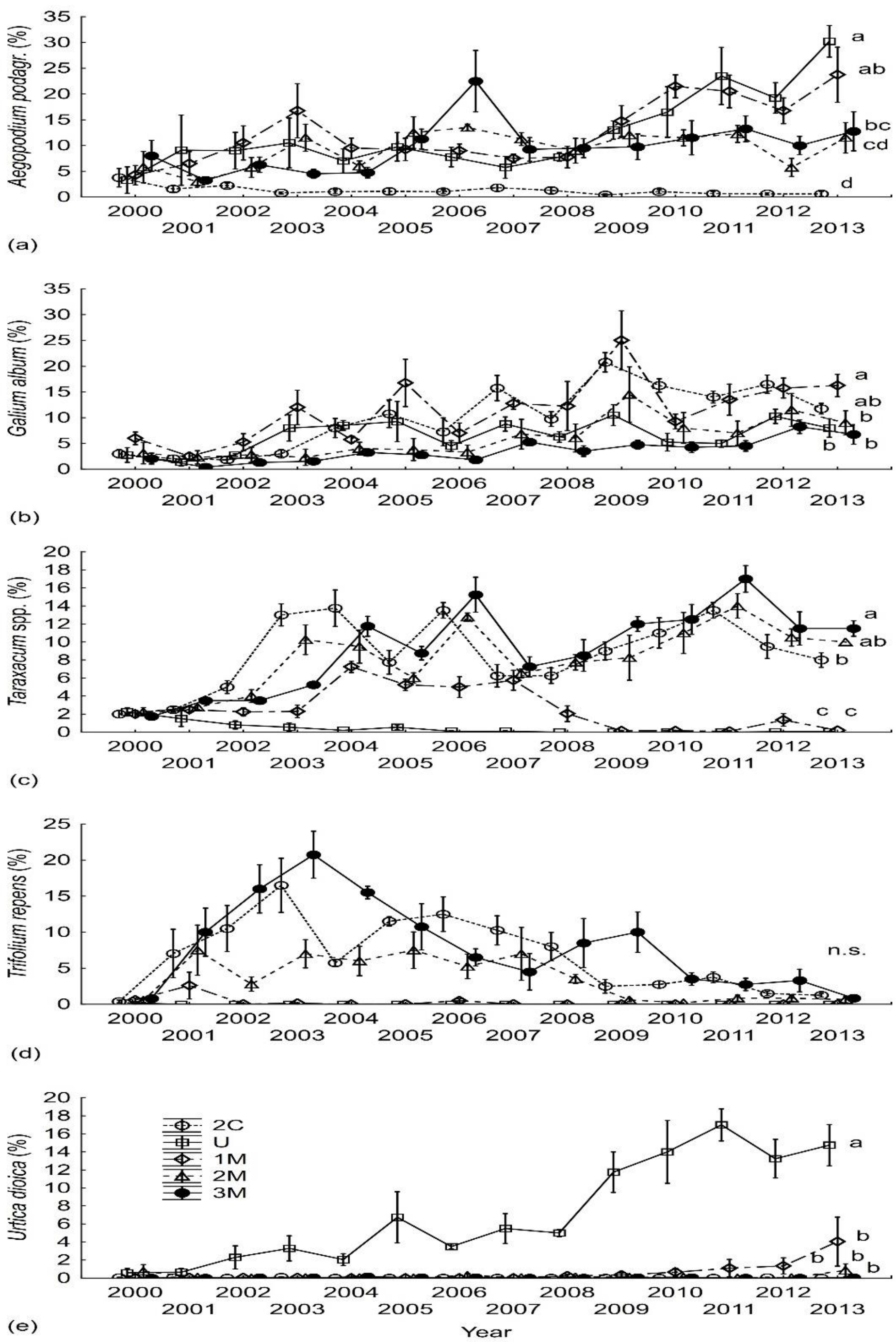

Figure 4. Changes in mean cover values of the dominant forb species – (a) *Aegopodium podagraria*, (b) *Gallium album*, (c) *Taraxacum* spp., (d) *Trifolium repens* and (e) *Urtica dioica* in investigated treatments between 2000-2013. Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatment abbreviations (U, 2C, 1M, 2M and 3M) are as explained in Figure 1.

The initial cover of tall graminoids varied from 52% to 62% at the start of the experiment, and it decreased regardless of treatment during the first four years. Subsequently, there were fluctuations but without any apparent stable trend for the particular treatments. However, the lowest cover of tall graminoids was recorded in the U treatment after 13 years of the experiment (Supporting Information Figure S2a). At the start of the experiment, the cover of short graminoids was very low (maximum of 0.5%). In later years, there was a tendency for an increase in short graminoids in the multiple-managed treatments (2C, 2M, 3M), whereas their proportion remained close to zero in the U and 1M treatments (Supporting Information Figure S2b). The initial cover of tall forbs varied between 13 and 22%, and we observed a progressive increase in the U and 1M treatments. There was a relatively stable cover of tall forbs under all the multiple-managed treatments (2C, 2M, 3M), the lowest proportion being recorded in the final year of the experiment (Supporting Information Figure S2c). On the other hand, the proportion of short forbs decreased in the U and 1M treatments, whereas it increased in the multiple-managed treatments (2C, 2M, 3M) (Supporting Information Figure S2d). The tall/short species ratio was < 6 in all of the multiple-management treatments (2C, 2M, 3M) over the duration of the experiment. After 2008, there was a steep increase in the tall/short species ratio; the highest value of the ratio was in treatment 1M after 13 years of the experiment (Supporting Information Figure S2e). Time and treatment, as well as their interactions, significantly affected proportions of species as classified by C-S-R functional strategies (competitors, stress tolerators and ruderals) (Table 2). Species characterized by C strategy predominated in all treatments, with proportions higher than 70%, and the C strategy was the one that characterized many of the plant species in the U and 1M treatments (>85%). The lower proportion of C strategy was revealed in both of the multiple mulching treatments (2M, 3M) and the lowest in the 2C treatment. Conversely, U and 1M treatments were least favourable for plant species with S and R strategies. The proportion of S and R strategies was similar and inverse to the C strategy (Supporting Information Figure S3) through the course of the experiment, with a differentiation between treatments in theneighth year of the experiment. The highest value was in the 2C treatment and the lowest the U treatment in after 13 years of the experiment.

The EIV for nutrients, light and moisture were significantly affected by time, treatment and by time x treatment interaction (Table 2). After 13 years of the management imposed, EIV for nutrients were significantly lowest in the 2C treatment, and significantly highest in all of the mulched (1M, 2M, 3M) treatments (Supporting Information Figure S4a). In the final year of the experiment, the EIV for light was highest in the 2C treatment and lowest in the U treatment, while values were similar in all of the mulched treatments (Supporting Information Figure S4b). On the other hand, there were opposite tendencies recorded for EIV of soil moisture, differentiation between treatments from the year 2008 and the highest value was in the U treatment and the lowest in the 2C treatment after 13 years of the experiment (Supporting Information Figure S4c).

4.3 Discussion

Species richness and diversity

Similar to the findings reported from our previous mulching experiment, which was conducted in a *F. rubra* semi-natural meadow (Gaisler et al. 2013), in all managed treatments, there were only small differences in the number of species that had cover values higher than or equal to 1%. This supports the view that, within the range of treatments implemented, the general plant species composition is relatively stable and remains similar under different management regimes. However, plant species with cover values of less than 1% contributed approximately half of the species richness, and therefore, these species exerted a greater effect on species fluctuation than more abundant plant species (Figure 1). The total number of vascular plant species at the beginning of the experiment (about 30 per 24 m^2) was in the range commonly found in improved upland grasslands in the Czech Republic (Pavlů et al. 2012). Moreover, similar numbers of species have also been recorded for semi-natural grasslands (Pavlů et al. 2011). However, there is usually a higher proportion of weedy species (e.g. *A. podagraria*, *C. arvense*, *E. repens*, *U. dioica*) recorded in improved grasslands (Mikulka et al. 2009) as was also revealed in our experiment. A positive effect of cutting or multiple mulching, in comparison with abandonment, on plant species richness is a common finding and has been reported from several manipulative experiments concerning grassland management (e.g. Doležal et al. 2011; Mašková et al. 2009; Nadolna 2009). Similar to species richness, the H index was lowest in the unmanaged treatment at the time immediately after abandonment and also for the once-mulched treatments after eight years from the beginning of the study (Figure 1c). This is consistent with the results obtained in a long-term mulching study conducted in Arrhenatherion grasslands in Central Germany. Although that study was not conducted on improved grassland, the sward consisted mainly of grasses of agricultural value (*A. elatius* and *Alopecurus pratensis*) and was managed by two cuts and fertilized (Laser 2002).

In order to avoid the decrease in plant species diversity that occurs in the absence of agricultural utilization, mulching when performed at least twice a year can be recommended as a suitable alternative method of grassland management and be appropriate for different types of grasslands (Gaisler et al. 2013; Laser 2002; Römermann et al. 2009).

Plant species composition and functional group

Based on RDA analyses, both tested null hypotheses (A1– the temporal trend in the species composition is independent of the treatment, and A2–there are no directional changes in time in the species composition that are common to all treatments) were refused. The analyses revealed that the temporal trends in the species composition are dependent on the treatments, and directional changes in time in the species composition are common to all treatments. These expected results are common to long-term manipulative experiments concerning different managements (Gaisler et al. 2013; Pavlů et al. 2011; Pavlů et al. 2012). The management treatments that involved frequent defoliation enabled the spread of several short forbs that have prostrate, rosette or creeping growth habit (*T. repens, Taraxacum spp., P. lanceolata*), and this has been common result from grassland management experiments throughout Europe (e.g. Belsky 1992; Pavlů et al. 2011; Pavlů et al. 2012; Supek et al. 2017). However, not all short forbs behaved in a similar way. For example, the rapid increase in the shade-intolerant (Grime et al. 1988) T. repens from its initial negligible cover was followed by successional decrease under repeated defoliation managements. High variation in the cover of *T. repens* between years have also been reported in other long-term manipulative experiments (Gaisler et al. 2013; Pavlů et al. 2012) and are probably associated with nitrogen-driven grass dynamics (Herben et al. 2017). On the other hand, the cover of *Taraxacum spp*. Was maintained over the years by frequent defoliation, with relatively low seasonal fluctuations (Figure 4c); a similar result was also revealed in a long-term study conducted in the same region (Supek et al. 2017). Similar to the results obtained in the previous experiment on low soil-nutrient status Festuca rubra-dominated, species-rich grassland (Gaisler et al. 2013) our findings reported in the present study support the results of Wahlman and Milberg (2002) that *P. lanceolata* is increased under management with two cuts per year, the traditional cutting frequency in Central European temperate grasslands (Ellenberg, 2009). Although G. album is known to be a species that succeeds in unmanaged or infrequently managed grasslands (Gaisler et al. 2013; Pavlůet al. 2007; Stránská 2004), its cover in all of the managed treatments in our experiment was equal to or higher than in the U treatment (Figure 4b). This is probably connected with the greater competitive ability of *U. dioica, A. podagraria, F. rubra and E. repens* than *G. album* in the U treatment, especially if there is an adequate level of plant available nutrients in the soil (Pavlů et al. 2016). Further treatments with repeated application of management operations during each season (whether as cutting or mulching) significantly reduced the cover of these tall weedy forbs, which are known to be sensitive to frequent defoliation (Pavlů et al. 2007; Přikrylová 2006). Furthermore, nutrient concentrations in the soil in these managed treatments, especially in the 2C treatment, were low relative to the elevated nutrient demand of these species. The survival and spread of short graminoids (*Agrostis capillaris, Anthoxanthum odoratum, Luzula sp.*) are usually associated with a management of repeated defoliation, particularly in nutrient-poor acid soils (Gaisler et al. 2013; Pavlů et al. 2007). In the present experiment, therefore, in which both soil pH and plant-available nutrients were relatively high, their cover was low regardless of the treatment imposed. The group of tall graminoids comprised several dominant grasses (*A. elatius, H. lanatus, F. rubra, E. repens* and *D. glomerata*) and these showed different responses to the management applied (Supporting Information Figure S2a). The sown grass D. glomerata was supported by all of the managed treatments, whereas for *E. repens* unmanaged or infrequently managed treatments were the most favourable. *Arrhenatherum elatius* was the grass with the highest cover in the once-mulched treatment, whereas for *H. lanatus* it was two cuts per year that appeared to be the most favourable management (Figure 3a, e). Although *H. lanatus* usually prefers fertile soils (Grime et al. 1988), its highest occurrence in our experiment was in the two-cut treatment combined with the lowest soil nutrient level. It seems likely that its preference for unshaded habitats (Grime et al. 1988) in mown meadows (Hejcman et al. 2010) is more crucial for this species than the soil nutrient status. The increased cover of *D. glomerata* in all of the managed treatments (Figure 3d) was probably a result of previous reseeding with the grass-clover mixture which included seed of this grass, as well as the subsequent and ongoing defoliation management. A gradual decrease in the amounts of *D. glomerata* in the unmanaged treatment could be connected to the low ability of this early growing tussock grass to sprout through thick layers of plant litter on the sward surface, particularly in early spring. Further, *D. glomerata* could have been suppressed by more productive species over the duration of the vegetation period (e.g. *A. podagraria, U. dioica and E. repens*). At the beginning of the experiment, *F. rubra* covered about 5% in all treatments and its development in subsequent years was supported by the unmanaged treatment only (Figure 3), similar to the findings reported in a previous study conducted in the same region (Pavlů et al., 2012). However, because *F. rubra* has high plasticity, it can be found in temperate grasslands under a wide range of management regimes (Gaisler et al. 2013; Grime et al. 1988; Krahulec et al. 2001; Pavlů et al. 2007; Pavlů et al. 2011) and nutrient levels (Hejcman et al. 2014; Honsová et al. 2007; Kidd et al. 2017).

Results on the tall/short species ratio revealed that the greatest changes occurred in unmanaged and in once-mulched plots after eight years of the treatment introduction (Supporting Information Figure S2e). This shows the high resilience of Central European temperate grasslands to contrasting types of defoliation management and that shifts to different sward structures can be recognized only after several years, as was also revealed in the Oldřichov Grazing Experiment (Pavlů et al. 2007). Plant species that share C strategy components in the C-S-R plant strategy theory of Grime et al. (1988) were the most thriving group in the experimental grassland of this study. This applied to all the experimental treatments but the proportion of plant species that share C strategy components increased with decreasing level of management intensity (Supporting Information Figure 3). This confirms previous results (Pavlů et al. 2010) that a higher percentage of C strategy species occurs in unmanaged grasslands than in grassland cut annually. The proportions of S and R strategists during the experiment were inverse to the proportion of C strategists, and cutting with removal of biomass was the management that was most favourable for plant species with S and R strategies. This also confirmed results from the previous mulching study conducted in *F. rubra* grassland (Gaisler et al. 2013). Similarly, to the tall/short species ratio, the C-S-R signatures differed most notably between treatments after eight years of the study. In comparison with the tall/short species ratio, the C-S-R signature for C, S and R strategy could even reflect differences between particular multiple-managed treatments.

EIV for nutrients reflected soil nutrient depletion in the two-cut treatment in this experiment (Pavlů et al., 2016). However, the results of EIV for nutrients for the other treatments were less closely related to the results of the laboratory analyses of soil nutrients, as they were originally related to biomass productivity of the sites (Schaffers and Sýkora 2000). The EIV for light and moisture showed clear distinctions between unmanaged and two-cut grassland, but they could not discriminate between the different mulching-frequency treatments. Overall, it appears that EIV for nutrients, light and moisture (Ellenberg et al. 1992) may provide good indirect predictors of ecological site conditions over the long term under different management practices. Our previous study concerning mulching in *F. rubra*-dominated grasslands with low nutrient status (Gaisler et al. 2013) showed that multiple mulching over a period of 12 years affected most plant species similar to that of cutting twice a year. Therefore, mulching performed at least twice a year may be recommended as an alternative management to traditional two-cut management, without any detrimental effects on plant species richness. Similar results have also been described for different low-productive grasslands in many European regions (Laser 2002; Moog et al. 2002; Römermann et al. 2009). Despite the very small differences between treatments in plant-available nutrients revealed in the previous study on this experiment (Pavlů et al. 2016), the detailed vegetation analyses reported in this paper showed that the long-term mulching management can reduce species richness and increase the proportion of weedy species. Therefore, it is not appropriate as a management option for previously intensified grasslands where the soil pH and nutrient status is adequate for agricultural utilization.

4.4 Conclusions

The unique value of this study is that it focuses on the effects of mulching versus other treatments on previously improved agricultural grassland, and particularly so given the relatively long period of the investigation. The main finding was that mulching, even if applied on multiple occasions in each growing season, cannot fully substitute for the traditional two-cut management in upland improved meadow without losses of plant species richness and diversity. The key changes in sward structure occurred after eight years, in the unmanaged and mulched-once- a year treatments, and were associated with an increase in the tall/short species ratio and the C-S-R signature component for the C strategy. The tall/ short species ratio could provide a convenient simple predictor for detecting structural changes in the sward, as affected by management intensity over time, which is easier to apply than the more detailed measures of changes in cover of individual plants, functional groups, species, or in the number of plant species. Mulching performed once a year in improved upland meadows encourages the spread of weedy species and the decline in species richness in a similar way to that of no management. Therefore, mulching may be considered useful only as a means of preventing the encroachment or invasion of improved upland grassland by shrubs and trees.

Acknowledgments

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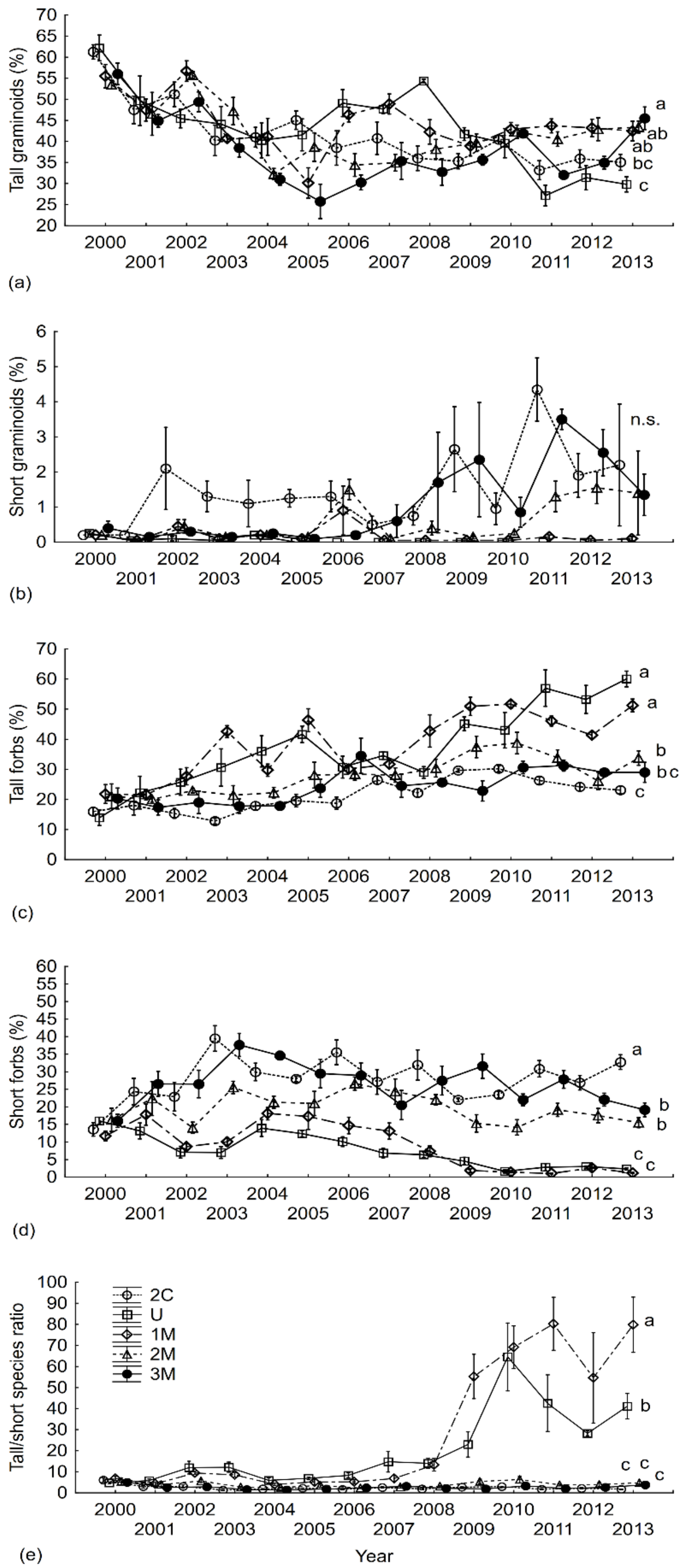
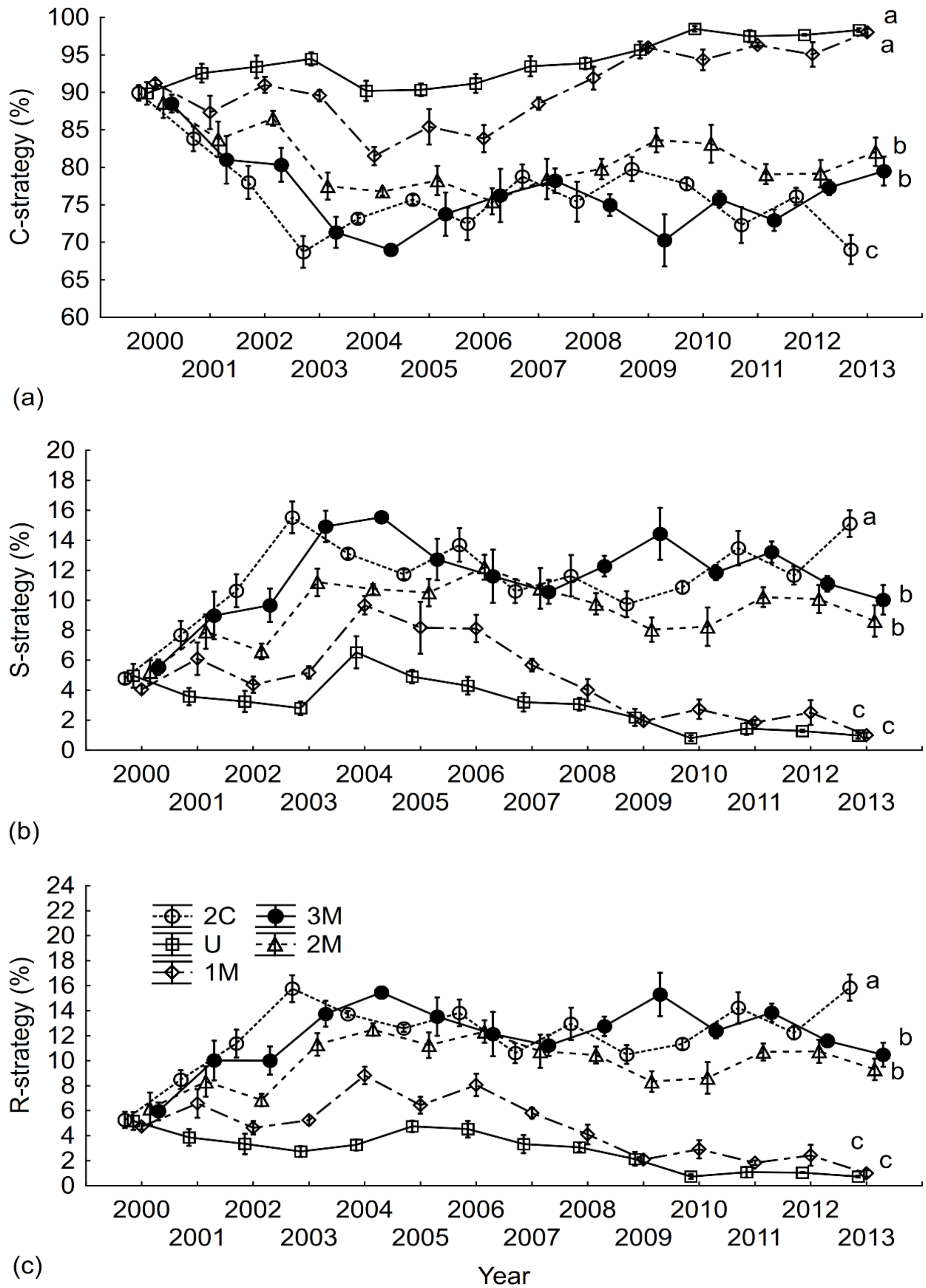
Supporting information Table 1 (S1)
Plant species recorded in the experiment grouped into functional groups. Weedy species in grasslands according Mikulka *et al.* (2009) are marked by **W** after the Latin name of plant species.

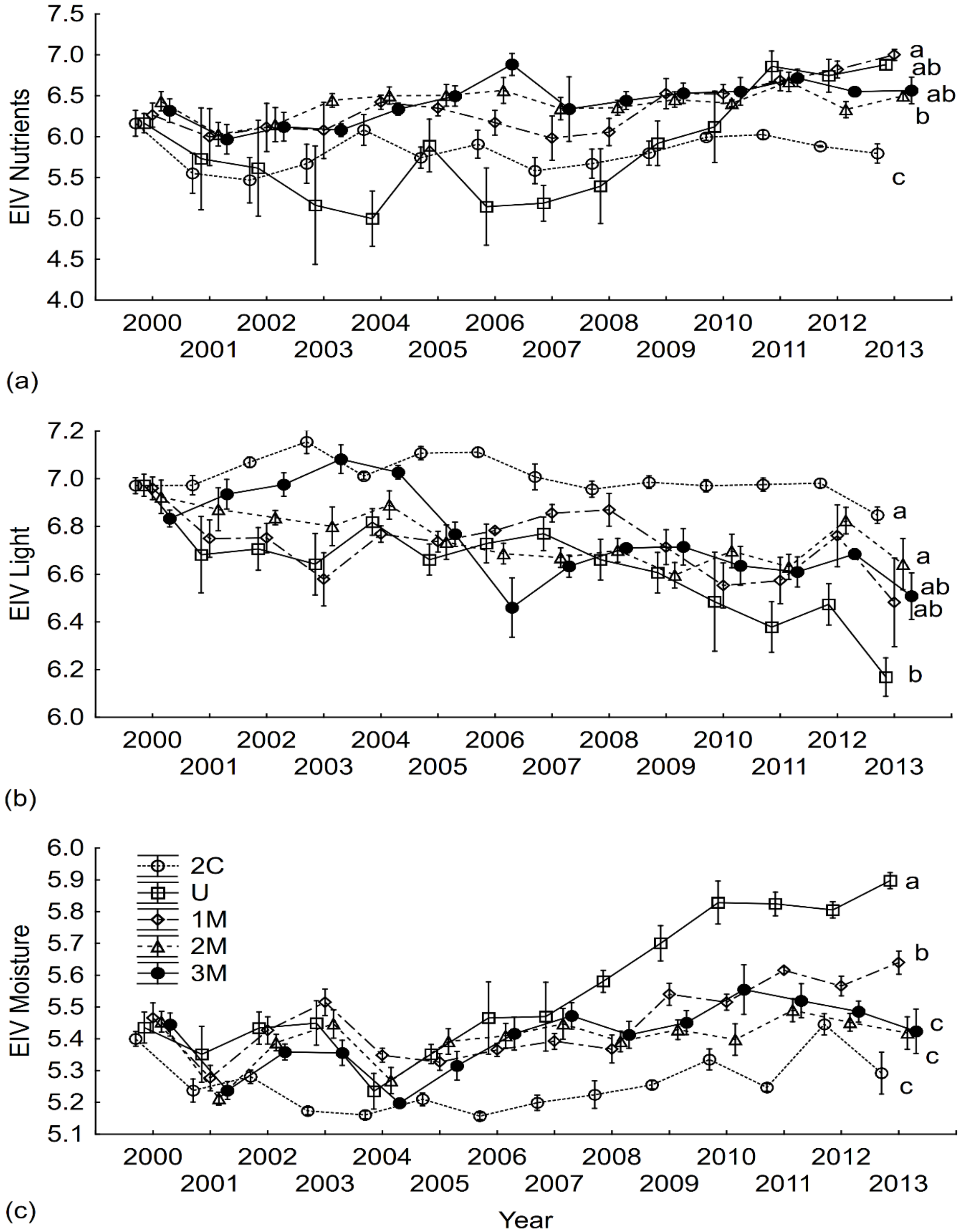
Tall graminoids	**Short graminoids**	**Tall forbs**	**Short forbs**
Alopecurus pratensis	*Agrostis capillaris*	*Achillea millefolium*	*Alchemilla* sp.
Arrhenatherum elatius	*Anthoxanthum odoratum*	*Aegopodium podagraria* **W**	*Bellis perennis*
Dactylis glomerata	*Luzula* sp.	*Angelica sylvestris* **W**	*Campanula patula*
Elytrigia repens **W**		*Anthriscus sylvestris* **W**	*Cardamine pratensis*
Festuca pratensis		*Artemisia vulgaris* **W**	*Cerastium holosteoides*
Festuca rubra agg.		*Bistorta major*	*Hylotelephium maximum*
Holcus lanatus		*Cirsium arvense* **W**	*Hypericum maculatum* **W**
Holcus mollis		*Cirsium palustre* **W**	*Hypochaeris radicata*
Poa pratensis		*Crepis biennis*	*Leontodon autumnalis*
Poa trivialis		*Galeopsis tetrahit* **W**	*Leontodon hispidus*
Trisetum flavescens		*Galium album*	*Lotus uliginosus*
		Heracleum sphondylium **W**	*Lychnis flos-cuculi*
		Lathyrus pratensis	*Lysimachia nummularia*
		Leucanthemum vulgare	*Myosotis* sp.
		Pilosella sp.	*Plantago lanceolata*
		Ranunculus acris	*Plantago major*
		Rumex acetosa	*Prunella vulgaris*
		Rumex obtusifolius **W**	*Ranunculus repens*
		Solidago canadensis **W**	*Stellaria graminea*
		Tanacetum vulgare **W**	*Taraxacum* spp.
		Urtica dioica **W**	*Trifolium hybridum*
		Vicia sepium	*Trifolium pratense*
			Trifolium repens
			Veronica arvensis
			Veronica chamaedrys
			Veronica serphyllifolia
			Vicia cracca



Supporting information Figure 2 (S2). Changes in mean cover of species grouped into the main functional groups – (a) tall graminoids, (b) short graminoids, (c) tall forbs, (d) short forbs and (e) tall/short species ratio in the investigated treatments between 2000-2013. Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatment abbreviations (U, 2C, 1M, 2M and 3M) are as explained in Figure 1.



Supporting information Figure 3 (S3). Changes in mean C-S-R signature (Grime *et al.*, 1988) for plants with (a) C, (b) S and (c) R strategy in the investigated treatments between 2000-2013. Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatment abbreviations (U, 2C, 1M, 2M and 3M) are explained in Figure 1.



Supporting information Figure 4 (S4). Changes in mean Ellenberg indicator values for (a) nutrients, (b) light and (c) moisture in the investigated treatments between 2000-2013. Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatment abbreviations (U, 2C, 1M, 2M and 3M) are explained in Figure 1.

CHAPTER FIVE

Effects of grazing and dung on nutrient level in herbage and soil

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Abstract

Faeces deposited by grazing animals are a key driver affecting sward structure and nutrient cycling in pastures. Therefore, the effects of grazing intensity on nutrient concentrations in herbage and soil under sward-height of patches were studied in an upland area in the Czech Republic. Three types of tall sward-height patches of were identified: (i) patches with faeces under intensive grazing; (ii) patches with faeces under extensive management; (iii) and patches with no faeces under extensive management. They were compared with frequently grazed swards under intensive and extensive grazing management. Analyses indicated no significant effect of different types of patch on soil available nutrients. Herbage from the different types of patches differed significantly in concentrations of N, P, K, Ca and Mg. The highest nutrient concentrations of P (4.5 g kg-1) and K (22.1 g kg-1) in the dry matter (DM) herbage were in patches with faeces under intensive grazing. The highest values for DM standing biomass, DM content, and sward height were detected in tall patches under extensive grazing. It is likely that nutrients deposited in heifer faeces were taken up by the plants, and therefore nutrient enrichment of soil was very low.

Keywords: heifers grazing; faeces; pasture vegetation; grazing intensity

5. Introduction

Grazing affects the structure of plant communities by influencing patch scale, patch type and patch stability. A moderate grazing intensity can result in a heterogeneous sward structure with spatial variations in height, which in turn affects the floristic composition and structure of plant communities (Tallowin et al. 2005). Selective defoliation, which is mainly due to dietary choice, is one of the main mechanisms by which grazing animals contribute to sward heterogeneity. First, it changes the competitive advantage among plant species through the selective removal of plant biomass (Bullock and Marriot 2000); secondly, it opens spaces for gap-colonizing species; and thirdly, there is contamination of the sward surface by the animals' dung and urine and this decreases the amount of forage available for grazing (Bokdam 2001). Furthermore, as the level of contamination increases there is increased rejection by grazing animals especially in the vicinity of dung pats (Forbes and Hodgson 1985). This rejection could persist over periods of several weeks, or even several years, as the time for complete decomposition of the dung pats is dependent on climatic conditions, water content of the dung and activity of the soil fauna (Dickinson and Craig 1990). Dung deposition, in combination with other grazing-related effects such as trampling, is an important factor that can explain the structure of vegetation in pasture (Kohler et al. 2004). It also has a significant effect on the chemical status of the soil and serves as a potential source of available nutrients for plants (Aarons et al. 2004).

We can distinguish the different types of patches resulting from grazing into frequently grazed areas that have little or sometimes no vegetation cover on the soil surface, and ungrazed or rejected areas dominated by mature plant species (Ren et al. 2015). Selection of whether patches are grazed or neglected also depends on the visual cues of the herbivore, which indicate forage quality or quantity, and the height of the patch itself (Wallis DeVries et al. 1999). In general, cattle graze shorter (< 10 cm) herbage patches in preference to taller (>10 cm) patches, which are mostly left ungrazed as their biomass is usually of lower feed value. This differentiation of patches into short and tall height is commonly observed in temperate grasslands (Ludvíková et al. 2015; Sahin Demirbag et al. 2009). Cattle also avoid areas with tall-stem herbage where the leafy components of the sward are difficult to graze (DeVries and Daleboudt 1994) and also areas that have been contaminated by faeces (Dohi et al. 1999; MacDiarmid and Watkin 1972b). There is also a tendency for selective grazing to increase over the course of the grazing season (Dumont et al. 2011).

Several studies have been conducted that have focused on the effects of dung patches in relation to botanical composition and nutrients, including studies in the UK (MacDiarmid and Watkin 1971; 1972a), Australia (Aarons et al. 2009) and USA (Teixeira et al. 2012; White-Leech et al. 2013). However, there has been little research that has focused on patches of different heights in swards in terms of the concentrations of nutrients in the herbage and in the soil, particularly for the upland grasslands of Central Europe. A study at our experimental site (Oldřichov Grazing Experiment) found that grazing intensity was the main driver affecting floristic composition of grasslands dominated by *Agrostis capillaris* (Ludvikova et al. 2015). Further, moderate and tall patches had similar botanical composition under a given stocking density, but the short patches showed different results than the other patches, especially under extensive grazing, and showed more botanical homogeneity under intensive grazing.

Therefore, our goal was to determine the effects of different intensities of grazing by heifers on the nutrient concentrations in the soil under tall sward-height patches and in the herbage of *A. capillaris* grassland. We hypothesized that the presence of faeces, deposited by heifers, on tall sward-height patches would significantly affect the amount of nutrients in the soil and consequently in the herbage of *A. capillaris* grassland. Within this context, we aimed to answer the following questions: (i) what is the effect of the presence of faeces on nutrient concentrations of soil beneath tall sward-height patches under intensive and extensive grazing management? (ii) does the presence of faeces on the surface beneath tall sward-height patches affect dry standing biomass yield, dry matter (DM) content, dead biomass, and nutrient concentrations in the herbage? and (iii) is there any relationship between soil nutrient concentrations and herbage nutrient concentrations under the tall sward-height patches?

5.1 Materials and Methods

Study Area

The study site "Oldřichov Grazing Experiment" is located in the Jizerské hory Mountains (Jizera Mountains) in the northern Czech Republic, 10 km north of the city of Liberec (50°50.34′ N, 15°05.36′ E) in the village of Oldřichov v Hájích. The experimental site was established in 1998 and has a mean annual temperature of 7.2 °C and an average annual precipitation of 803 mm (Liberec Meteorological Station); the altitude is 420 m above sea level. The site has a medium deep brown soil (Cambisol) with 10-15 cm and is underlain by granite bedrock.

The sward on the experimental site has a high diversity of plant species, with about 24 vascular plant species per m^2. The dominant species are *Agrostis capillaris, Festuca rubra* agg., *Trifolium repens* and *Taraxacum officinale* (Ludvíková et al. 2015; Pavlů et al. 2007). The productivity of the pasture is 2-4 t DM ha^{-1} $year^{-1}$ (Pavlů et al. 2007).

Experiment Design and Plot Management

The experimental site was established as two completely randomized blocks (Pavlů et al. 2006; Pavlů et al. 2007). Each block consisted of four paddocks with different grazing regimes (treatments) and each experimental paddock was approximately 0.35 ha (Ludvíková et al. 2015). For this study we selected two paddocks, in each block, with two contrasting long-term levels of grazing intensity: (i) extensive grazing (EG), with a mean target sward surface height of greater than 10 cm; and (ii) intensive grazing (IG) with a mean target sward surface height of less than 5 cm. This was achieved by increasing or decreasing the area available for grazing by moving fences with a set number of stock per plot for IG or EG. Stocking density was adjusted through the grazing season accordingly. The stocking rate recorded in September was about 1000 kg and 500 kg live weight per ha under IG and EG respectively. All paddocks were grazed under continuous stocking with young heifers (Czech Fleckvieh) of initial live weights of about 200 kg, from early May until late October.

Data collection of herbage and soil

Sward height measurement, herbage biomass and soil samples were taken at the late of grazing season on 18 September 2013, when tall sward-height patches were fully developed. To study the effect of grazing intensities on nutrient concentrations in the soil and the herbage, we identified three types of tall sward-height patches: i) IG_TF - tall patches in IG with presence of residual spring faeces; ii) EG_TF - tall patches in EG with presence of residual spring faeces; iii) EG_T0 - tall patches in EG without presence of residual spring faeces. (For details see Table 1). For the IG grazing intensity we were unable to find any presence of the tall sward-height patches without faeces. Tall patches were compared with frequently grazed patches in intensive (IG_C) and extensive (EG_C) grazing.

Four soil and herbage samples were then randomly taken in each of two paddocks in block. Visual identification was carefully done on the EG plots for patches, since all patches had a canopy height of > 10 cm. It was possible to differentiate between patches surrounded by, or partly covered with faeces, from other patches that were free from faeces or even not grazed. The partly decomposed spring faeces were typically about 5-8 cm in diameter. The mean diameter of recently deposited dung pads is around 25-30 cm in diameter. The selected patches with faeces were compared and proved with artificially established spring faeces and were sampled only in case of similarity of decomposition stage

The mean values of nutrient concentrations in the spring faeces of heifers regardless treatment were 21.1, 6.6, 7.7, 18.5, 4.3 g kg^{-1} for N, P, K, Ca and Mg, respectively (V. Ludvíková unpublished data). Spring fresh faeces were 20-30 cm in diameter and weighed about 1 kg, with about 15-20% DM content.

To characterize sward height distribution in IG and EG 100 measurements were taken along a transect in four paddocks of both treatments (400 measurements in total). During each measurement there was a visual identification of the presence of faeces and of non-grazed sward.

The sward-height of the sward and the patches was measured using a rising plate meter (Correll et al. 2003). Using a circular ring of 30 cm in diameter on each type of patch the proportion (as %) of dead plant biomass was assessed by visual observation; herbage biomass was then cut to ground level. The harvested herbage was weighed fresh, oven dried at 80 °C and the DM content and dry matter standing biomass (DMSB) were determined. Under each patch, faeces, if present, were removed and soil samples were taken from the upper 10 cm of the soil profile using an auger and the biomass residues and roots were removed. The soil samples were air dried and then ground to pass a 2 mm sieve.

From the herbage used to determine the DM content, the concentrations of N, P, K, Ca and Mg were determined. The herbage concentrations of N, P, K, Ca and Mg were determined after digestion of DM herbage in aqua regia by inductively coupled plasma–optical emission spectrometry. Plant available P, K, Ca, Mg were extracted by Mehlich III (Mehlich 1984). Total nitrogen (N_{tot}) was determined by the Kjeldahl method and organic C content (C_{org}) by means of colorimetry (AOAC 1984). Determination for pH/Cal_2 was done by using acidometer. All chemical analyses for soil and herbage were performed in an accredited laboratory at the Crop Research Institute in Chomutov.

Data analysis

A linear mixed-effects model with fixed effects of treatment and random effect of block was used to analyse the effect of different type of patches on concentrations of each individual nutrient in the soil and the herbage - DMSB, SH, DM content, and proportion of dead biomass. Block was use as covariate with random effect in all performed analyses. Post hoc comparison using the Tukey HSD test was applied to identify significant differences among different types of patches. Selected soil chemical properties, sward characteristics were log transformed to improve normality and homogeneity. Finally, linear regression analysis was used to identify the relationship between plant available nutrients in the soil and the nutrient contents in the herbage. All univariate analyses were performed using Statistica 13.1 (Dell Inc. 2016).

A redundancy analysis (RDA) in CANOCO 5 (ter Braak and Šmilauer 2012) was used to reveal the effect of different types of patches on all nutrients in the soil and the herbage and other sward characteristics. Each analysis was performed with 999 permutations. Species data were log-transformed ($y = \log10 (y + 1)$). The blocks were treated as covariables. An ordination diagram, constructed by the CANOCO 5 program, was used to visualize the results of the RDA analysis.

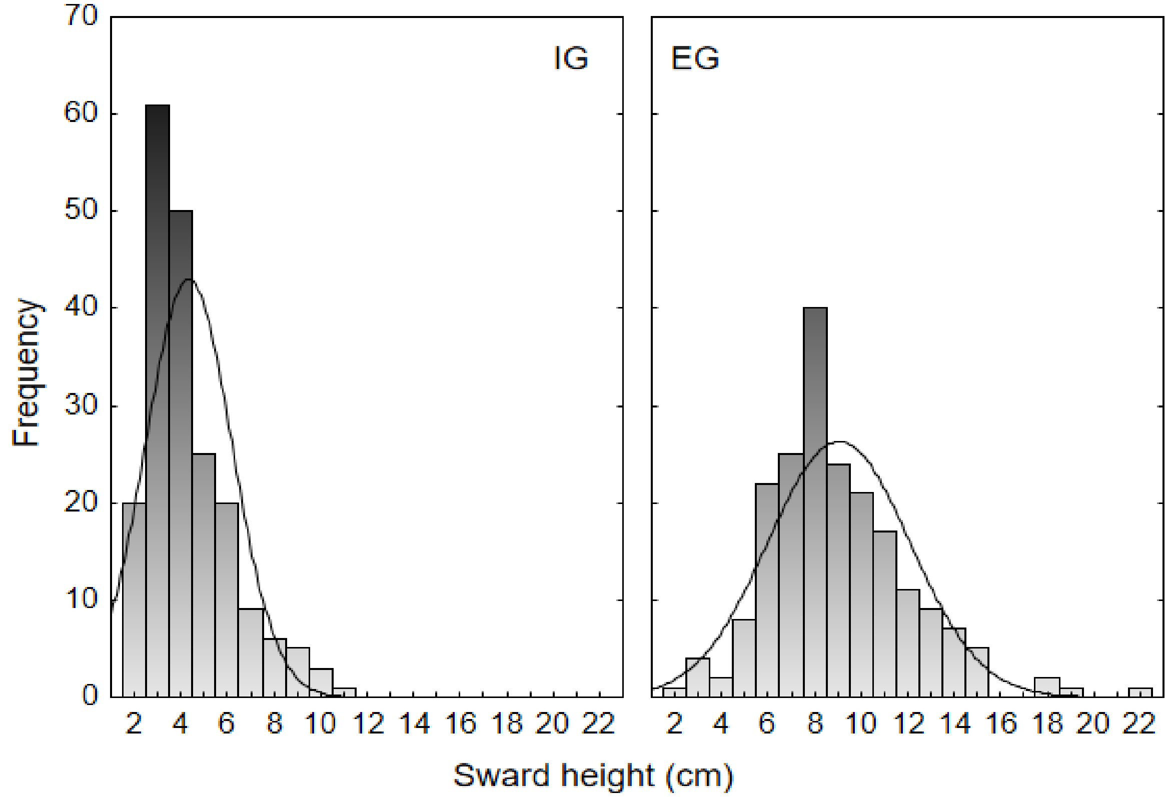

Figure 1. Frequency distribution showing sward height (cm) variation in intensive grazing (IG) and extensive grazing (EG) treatments.

5.2 Results

Sward Characteristics

Frequency of distribution of sward heights during the sampling under IG and EG is shown in Figure 1. The proportion of presence of faeces in tall patches was under IG 5.0% (IG_TF) and under EG 4.5% (EG_TF) (Table 1). The highest values for SH, DM content, and DMSB were found under EG_T0 and EG_TF patches, and the highest values for dead biomass was found under EG_T0 and EG_C, whereas the lowest values were found under the IG_C patches (Table 2 3). Generally, sward characteristics reflected grazing intensity in the order EG_T0 > EG_TF > EG_C > IG_TF > IG_C.

Table 1. Description of the sward height patches and their management.

Patch abbreviation terms used in text	Grazing management	Target average sward height	Patch type	Faeces presence	Stocking rate (kg live weight per ha)	Frequency of patches occurrence
IG_C	Intensive grazing	< 5 cm	Frequently grazed	-	1000	0.95
IG_TF	Intensive grazing	< 5 cm	Non-grazed or infrequently grazed tall sward patches > 10 cm	+	1000	0.05
EG_C	Extensive grazing	>10 cm	Frequently grazed	-	500	0.925
EG_TF	Extensive grazing	>10 cm	Non-grazed or infrequently grazed tall sward patches > 10 cm	+	500	0.045
EG_T0	Extensive grazing	>10 cm	Non-grazed or infrequently grazed tall sward patches > 10 cm	-	500	0.03

Table 2. Soil chemical properties under different sward height patches: pH/$CaCl_2$, N_{tot}, C_{org}, plant available (Mehlich III) concentration of P, K, Ca, Mg and C:N ratio in 0-10 cm layer. Numbers represent average values of patches, ± values represent standard error of the mean (SE). F-ratio = F-statistics for the test of a particular analysis, P-value = corresponding probability value. Significant differences (P <0.05) between patches according to Tukey's Post-hoc test are indicated by different letters in the row. Abbreviations for the type of patches see Table 1.

Soil chemical properties	Patches					*F*-ratio	*P*-value
	Tall sward height patches			Frequently grazed			
	IG_TF	EG_TF	EG_T0	IG_C	EG_C		
pH/$CaCl_2$	5.49±0.0a	5.62±0.20a	5.27±0.06ab	4.91±0.07b	5.06±0.07b	7.8	<0.001
N_{tot} (mg kg^{-1})	5066±101	5041±171	4886±187	4876.80±190	5068.23±255	0.27	0.897
P (mg kg^{-1})	53.72±7.37	41.40±4.31	47.24±6.78	51.36±6.82	52.36±7.15	0.56	0.693
K (mg kg^{-1})	226.42±38.23	192.12±15.97	191.77±14.63	156.47±18.69	173.14±18.96	1.49	0.228
Ca (mg kg^{-1})	1910±123	2016±192	1830±131	1470±111	2036±142	2.52	0.06
Mg (mg kg^{-1})	178.46±16.31	166.23±22.70	152.38±16.23	113.60±12.52	159.93±14.96	2.21	0.089
C_{org} (mg kg^{-1})	49838±1047	53800±1528	52563±1955	48655±2466	54892±2736	1.66	0.181
C:N	9.84±0.32c	10.69±0.32bc	10.77±0.32bc	11.34±0.26ab	12.65±0.614a	11.54	<0.001

Nutrient concentrations in the soil and in the herbage

Type of patch did not show any significant effect on the concentrations of N_{tot}, C_{org} and plant available nutrients P, K, Ca and Mg in the soil (Table 2). The lowest pH/$CaCl_2$ was recorded for the IG_C, EG_C and the highest value was under IG_TF, EG_TF and EG_T0. The highest C:N ratio was found under the EG_C patch, whereas the lowest was under the IG_TF, EG_TF and EG_T0 patch.

The presence of faeces increased P and K concentrations in herbage in IG_TF. The highest Ca concentration was under IG_C, whereas the highest N and Mg concentrations were found under IG_C and IG_TF patch (Table 3). However, no effect of the presence of faeces on N, P, K, Ca and Mg concentrations in herbage was found in all patches sampled under the extensively grazed pasture. The highest N:K and N:P ratios in the herbage were found under both frequently grazed patches regardless of the grazing intensity (IG_C, EG_C). No significant differences were found in the K:P ratio between patches. The lowest Ca:P ratio was revealed in IG_TF, but there were no differences between other patches.

Generally, in the non-grazed or infrequently grazed patches there were higher total amounts of herbage nutrients per m^2. The total amount of herbage nutrients was in the order of EG_T0, EG_TF > IG_TF, EG_C > IG_C for N, P, K, Ca and Mg. The presence of faeces had no significant effect on total amounts of herbage nutrients per m^2 in tall sward-height of patches under extensive grazing (EG_T0, EG_TF) (Table 3).

Relationship between herbage and soil nutrient concentration

The RDA analysis showed a significant ($P < 0.001$) difference for the first ordination axis and all axes in the soil and the herbage nutrient concentration. The percentage of explained variability by the first and second ordination axes was 63.0 and 72.6 respectively. The higher herbage nutrient

N, P, K, Ca, Mg concentrations were associated with IG_TF and IG_C patches (Figure 2). On the other hand, higher values for SH, DM content, DMSB and cover of dead biomass were linked with both types of tall patches (EG_TF, EG_T0) on extensively managed sward patches, regardless of whether faeces were present. The regression analysis showed no relationship between concentrations of nutrients in the soil and in the herbage.

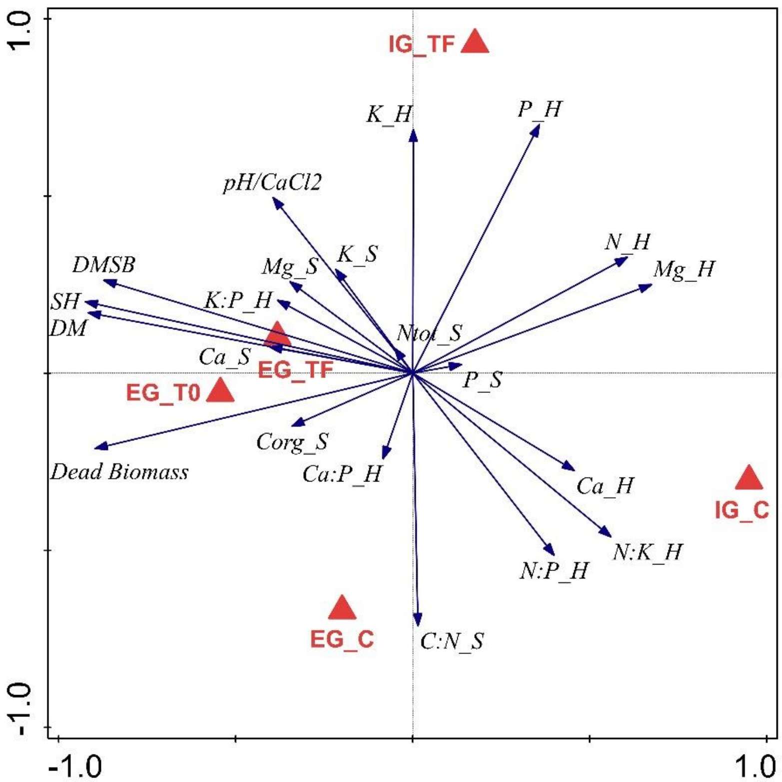

Figure 2. Ordination diagrams showing results from RDA analysis of herbage and soil nutrient concentrations. Abbreviations: Ca_H = calcium in herbage, Ca_S = calcium in soil, C_{org}_S = organic matter in soil, DM = percentage of dry matter content in herbage, DMSB = dry matter standing biomass, K_H = potassium in herbage, K_S = potassium in soil, Mg_H = magnesium in herbage, Mg_S = magnesium in soil, N_H = nitrogen in herbage, N_{tot}_S = total nitrogen in soil, P_H = phosphorous in herbage, P_S = phosphorous in soil, SH = sward height. Abbreviations for the type of patches see Table 1.

Table 3. Sward characteristics and herbage nutrient concentrations of different sward height patches. Numbers represent average values of patches, ± values represent standard error of the mean (SE). F-ratio = F-statistics for the test of a particular analysis, *P*-value = corresponding probability value. Significant differences (*P* <0.05) between patches according to Tukey's Post-hoc test are indicated by different letters in the row. Abbreviations for the type of patches see Table 1. Abbreviations for sward characteristic: DM = dry matter content, SH = sward height and DMSB = dry matter standing biomass.

	Patches						
Parameters measured	Tall sward height patches			Frequently grazed			
Sward characteristics	IG_TF	EG_TF	EG_T0	IG_C	EG_C	*F*-ratio	*P*-value
SH (cm)	10.00±0.46b	14±0.98a	15.37±0.98a	3.63±0.26c	10.38±0.63b	39	<0.001
DM (%)	18.09±0.68b	24.13±0.72a	27.41±1.27a	10.48±0.32c	18.53±1.14b	58.46	<0.001
DMSB (g m-2)	358.58±77.93b	548.29±57.42a	707.43±90.73a	79.03±8.18c	254.91±12.23b	47.37	<0.001
Dead biomass (%)	8.38±2.38c	24.38±2.58b	32.5±0.94a	1.63±0.26c	28.75±1.83ab	53.28	<0.001
Herbage nutrients							
N (g kg-1 DM)	30.65±2.96a	18.68±0.40cd	16.68±0.34d	25.49±0.67ab	22.56±0.39bc	21.48	<0.001
P (g kg-1 DM)	4.51±0.28a	2.75±0.08bc	2.40±0.09bc	2.96±0.05b	2.75±0.07bc	34.89	<0.001
K (g kg-1 DM)	22.06±1.66a	14.73±1.30b	11.87±0.63b	11.79±0.92b	12.53±0.68b	12.25	<0.001
Ca (g kg-1 DM)	6.14±0.37b	7.24±0.63ab	6.12±0.46b	9.14±0.70a	6.92±0.51ab	4.97	0.003
Mg (g kg-1 DM)	2.69±0.17a	1.97±0.15b	1.75±0.11b	2.84±0.19a	2.01±0.12b	11.41	<0.001
N:P	6.81±0.57c	6.83±0.20c	6.98±0.22bc	8.62±0.22a	8.27±0.32ab	6.82	<0.001
N:K	1.39±0.09b	1.34±0.11b	1.43±0.07b	2.28±0.22a	1.84±0.10ab	9.615	<0.001
K:P	4.97±0.41	5.41±0.54	4.97±0.28	3.98±0.29	4.56±0.26	2.229	0.086
Ca:P	1.38±0.08b	2.64±0.23a	2.54±0.15a	3.09±0.23a	2.52±0.18a	12.27	<0.001
Total amount of nutrients in herbage per area							
N (g m-2)	10.66±2.76b	10.30±1.18a	11.82±1.58a	2.01±0.20c	5.74±0.27ab	20.73	<0.001
P (g m-2)	1.52±0.27b	1.49±0.14a	1.72±0.26a	0.24±0.03c	0.70±0.04b	40.89	<0.001
K (g m-2)	8.10±2.33a	7.97±1.13a	8.42±1.19a	0.92±0.11b	3.22±0.26a	7.6	<0.001
Ca (g m-2)	2.16±0.44b	3.97±0.52a	4.37±0.73a	0.73±0.10c	1.77±0.16b	30.6	<0.001
Mg (g m-2)	0.95±0.20b	1.11±0.17a	1.23±0.17a	0.23±0.04c	0.51±0.04b	37.21	<0.001

5.3 Discussion

The type of patches had no effect on the concentrations of P, K, Ca, Mg, C_{org} and N_{tot} in the soil. This finding was in contrast to results from other studies that were directly focused on dung patches and which reported direct positive effects of dung-derived nutrients on the nutrient concentrations in the soil (Aarons et al. 2009; MacDiarmid and Watkin 1972a; Teixeira et al. 2012; White-Leech et al. 2013). This inconsistency in results between the present study and previous research might be attributed to nutrient movement through the soil sampling depth, or to differences among types of grassland ecosystem, grazing period, soil type, differences in plant species, and environmental factors (Patra et al. 2008; Gavazov et al. 2013; Yang et al. 2018). The site-specific conditions are underlined by results from an experiment conducted in the near vicinity of this experiment in which there was found to be a low residual effect of the previous inorganic fertilization in terms of nutrient concentrations in the soil and herbage (Pavlů et al. 2012a). Probably as a result of nutrient leaching in this type of soil. In addition, the failure to find an effect of faeces on soil nutrient concentrations was probably also affected by areas having nonvisible residual faecal and urine nutrients from a previous season.

The quality (nutrient content) and size of faeces deposited by heifers on the sward surface were among other factors likely to have affected our results. Based on the average amount of faeces, their nutrient concentrations and area of coverage, the amounts of nutrients applied in individual dung patches were calculated as follows: 40-60 g N m^2, 14-20 g P m^2, 16-25 g K m^2, 40-60 g Ca m^2 and 10-14 g Mg m^2 ha^{-1}. These values are approximately two times lower than those reported for cattle by Whitehead (2000), differences which may be explained by different types of grazed sward, supplementary feeding, weight of animals and breed. However, even these lower values represent a high input of nutrients applied on to a small area.

The higher values for SH, DM content and DMSB under EG_TF and EG_T0 compared with the IG_C, EG_C and IG_TF were associated with greater herbage maturity and greater proportion of dead plant biomass in the sward. Generally, in Central European upland grasslands the DM content in the herbage and DMSB increase as the sward ages (Pavlů 2015) and the proportion of dead plant biomass increases with extensification management (Pavlů et al. 2012b). These higher DMSB values in the SH of patches under extensive grazing relative to intensive grazing were also found in a previous study (Correll et al. 2003) conducted on this same *Agrostis capillaris*-dominant pasture. The higher N, P, K and Mg concentrations in the herbage revealed in the tall sward-height patches with faeces presence (IG_TF) can be explained by heavy over-fertilization caused by the heifers' faeces and urine, but higher concentration of Mg in the herbage was also found in the frequently intensively grazed sward. Higher concentrations of Mg and Ca in the herbage in a frequently intensively grazed sward was connected to there being a higher proportion of white clover (*T. repens*) and dandelion (*T. officinale*) in the sward (Ludvíková et al. 2015; Supek et al. 2017) as these prostrate herbs have been reported to have higher Mg and Ca concentrations in the herbage (Whitehead 2000; Pirhofer-Walzl et al. 2011). Therefore, Mg and Ca higher uptake could also be the reason for the tendency of lower Ca and Mg in the soil concentrations under IG_C patch. In general, the nutrient concentrations in the herbage of all SH of patches were within the range recommendation for the nutritional requirements of cattle (Whitehead 2000). Further, they were also in the scale revealed for this type of upland Central European grassland managed by grazing (Pavlů 1994; Pavlů and Velich 1998).

Herbage at early stages of maturity usually has higher nutrient of concentrations compared with the remainder of' the grazing season (Pavlů and Velich 1998; Pavlů 2015). On the other hand, there is a nutrient "dilution effect" with advancing growth and maturity (Duru and Ducrocq 1997; Pavlů

2015) and this may explain the lower mineral concentrations (N and Mg) in the herbage that were recorded in all patches under extensive grazing (EG_C, EG_TF and EG_T0). Furthermore, the presence of faeces in the infrequently grazed tall sward patches under extensive grazing management had no effect on nutrient concentrations in the herbage.

The lack of relationship between concentrations of nutrients in the soil and in the herbage from regression analyses might be due to uptake by the plant, as the data were expressed in terms of amount of nutrients in the herbage per unit of area. This finding is in accordance with Dickinson and Craig (1990), suggesting nutrient losses from the dung are not necessarily associated with increases in nutrients in the soil, and arguing that the nutrients might have been immediately used by the plants under the dung as soon as the nutrients were released from the dung. In addition, the results from the soil revealed no differences in nutrient concentrations, and the main differences among patches were in the nutrient concentrations in herbage. Presence of faeces in tall sward-height of patches (IG_TF, EG_TF) significantly increased biomass production in comparison with frequently grazed patches (IG_C, EG_C), which consequently contributed to higher nutrient accumulation in the herbage biomass. Therefore, the total amounts of herbage nutrients per m^2 can be connected not only with DMSB but also with the presence of faeces.

5.4 Conclusions

There was found no effect of the presence of faeces under intensive and extensive grazing management on the P, K, Mg, Ca, C_{org} and N_{tot} concentrations in the soil under the tall sward-height patches. It is likely that the nutrients deposited in the heifer faeces were taken up relatively quickly by the sward through vegetation season, and therefore soil enrichment was very low. The presence of faeces increased the P and K concentrations in the herbage in tall sward-height patches in intensively grazed pasture only, whereas no effect of faeces was found in extensively grazed pasture, which is likely due to a "dilution effect".

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CHAPTER SIX

Five years of chicken grazing on sown grassland and its effect on soil chemical properties and vegetation

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ABSTRACT

Chicken grazing (CG) has been discovered to be effective management for poultry meat production in grasslands worldwide. However, the effects of CG on soil quality of Central European grasslands are still not understood. The study was focused on the effects of CG on soil nutrient concentration under five years field study in sown improved grassland. Total N and plant available Mg were the only nutrients that were significantly increased during the study under CG. However, the study observed a reduction of sown high productive species, except *Festulolium* and increasing cover of weedy *Taraxacum officinale*. This five-year study showed that CG can increase nutrients concentrations in the soil and deteriorate the botanical composition of grassland.
Key words: Chicken pasturing; grasslands, soil chemical properties, plants characteristics,

6. INTRODUCTION

Chicken grazing (CG) or ‘pastured’ or ‘free range’ poultry is an alternative production system where birds are reared entirely on fenced-in pasture or small, open-air moveable pens and having daily access to fresh pasture. Unlike ruminants, chickens are omnivores that derive various foods (such as insects, fresh leaves, invertebrates, buds, grains and stones) from grasslands (Lomu et al. 2004), while causing minor harm to soil and plants (Su et al. 2018). Their inherent scratch-feeding mode promotes soil surface aeration and the incorporation of organic matter into the soil (Fukumoto 2009). Notably, free-ranging chicken production could increase labile manure to degraded soil, thereby enhancing plant growth in the poultry farmlands (Grichar et al. 2005).

Chicken grazing has been widely incorporated into other farm enterprises in some European countries (Berton and Mudd 2000), and it has not only demonstrated a cost-effective livestock production system but can also provide the sustaining environment for more household-farmers on their lands (Glatz et al. 2005, Sossidou et al. 2011). In addition, chickens have other advantages of controlling pests and spreading their manure to grassland ecosystems (Sossidou et al. 2011).

The zeal for farmers to use pasture raised chickens in improving soil quality and boost plants yield has increased in recent years, as has consumers' demands for pastured poultry products (Burbaugh et al. 2010, Hilimire 2011). The benefit of manure as a source of plant nutrients has long been identified, and chicken manure is a concentrated plant food containing two to three times as much nitrogen, three to five times as much phosphorus, and about the same amount of potassium and organic matter as other farm manures (McCall 1980, Zublena 1993). And apart from being a profitable source of plant nutrients, chicken manure is an essential soil conditioner, and it enhances the soil 's holding capacities of moisture and nutrient (McCall 1980).

Although, several authors have reported the high mineral content of poultry manure (Li et al. 2011, Sossidou et al. 2011, Kobierski et al. 2017, Su et al. 2018), yet some fewer studies have revealed that the poultry manure might have a negative effects on the ecosystem especially soil and water depending on the duration of the introduction (Mishra et al. 2015, Suchy et al. 2018) or the site's prevailing soil properties (Hanč et al. 2008).

In terms of manure quantity, estimates of the manure excreted by 1 000 birds per day (based on average daily live weights during the birds' production cycle) are approximately 120 kg for layer chickens, and 80 kg for meat chickens (Collins et al. 1999). One hen can produce 59 kg of manure per year which is about 0.161 kg per hen in a day (Bolan et al. 2010, McCall 1980. Though, there has been many studies on the fertilizer content values of poultry manure and litter (Adeli et al.

2010, Sainju et al. 2008, Tewolde et al. 2010, Edmeades 2003), yet there are limited publications regarding the effects of poultry farming especially live birds on soil fertility. We hypothesized that CG caused increased soil N while, preferential grazing by the chickens led to changes in botanical composition. Therefore, the study foced at determining whether soil from longer-term CG enhanced soil nutrient concentration in comparison with before grazing. In this context, the study addressed the following research question: Is there any significant relationship in the soil chemical properties before and after grazing?

6.1 MATERIALS AND METHODS

The chicken grazing experiment was conducted on 0.7 ha of experimental grassland at Netluky village, Czech Republic (150°2'21.344"N, 14°36'51.075"E). The altitude of the study site was 284 m a.s.l., the average annual precipitation was 591 mm and the mean annual temperature was 8°C. The soil was loamy clay with the following soil chemical properties before and during the experiment in the Spring 2013 and beyond (Table 1). Experimental site was sown by the grass/clover mixture (35 kg of seeds per ha) in early Spring in the year 2012. The mixture consisted with the following species: *Festuca pratensis* (c.v. Kolumbus) 20%, *Festuca rubra* (c.v. Levočskaá) 10%, *Lolium perenne* (c.v. Merlinda) 15%, *Poa pratensis* (c.v. Slezanka) 10%, *Trifolium pratense* ("Violetta") 20% and the intergeneric hybrid (c.v. Felina) 25%.

The chicken grazing was performed in floorless portable pen in the core of experimental grasslands of size 85 m x 25 m. For detailed specification of floorless portable pen and pens movement during grazing season see Skřivan et al. (2015). Before chicken grazing in each season, grassland was cut and hay harvested. The descriptions of chicken grazing management in the years 2013-2017 are shown in Table 2.

On the transect of 60 meters (main line of chicken grazing) fixed four points in distance of 2 meters were selected. The twelve (four points x three replication) soil samples were taken from the upper 10 cm of the soil profile using an auger around permanent network in Spring 2013 and after that in September (Autumn) 2013-2017. After sampling, the biomass residues and roots were removed, then the soil samples were air dried and then ground to pass a 2 mm sieve. Plant available P, K, Ca, Mg were extracted by Mehlich III (Mehlich 1984), total nitrogen (N_{tot}) was analysed by the Kjeldahl method and organic C content (C_{org}) by means of colorimetry (AOAC 1984). The soil pH-$CaCl_2$ was determine through a mixture of soil and 0.01 M $CaCl_2$ (Graham 1959, Schofield and Taylor 1955). All chemical analyses for the soil samples were performed in accredited laboratory at the Crop Research Institute in Chomutov.

The percentage cover (up to 100%) of all vascular species was assessed in the core of experimental grasslands 85 m x 25 m before soil sampling.

A linear mixed-effects model with fixed effect of year and random effect of replication was used to test differences of the soil chemical properties during the five years study. Post hoc comparison using the Tukey HSD test was used to identify significant differences among different sampling dates. The data met all requirements of ANOVA and all analyses were performed using Statistica 13.1 (Dell Inc. 2016).

6.2 RESULTS AND DISCUSSIONS

Significant differences were found in all the soil chemical properties monitored but the trend was not obviously defined across the years (Table 1). For example, in the second year of sampling (autumn 2014), pH values significantly decreased to 6.44 and this lower value lasted till the year 2017. Plant available P concentration significantly decreased below 100 mg kg^{-1} in the second year of study (2014), after that it increased again to 120 mg kg^{-1}. In the year 2017, the plant available P concentration had similar values as before the establishment of the experiment. The concentration of plant available K was relatively stable around 300 mg kg^{-1} after the decrease in the Autumn 2013 to 240 mg kg^{-1}. During the study years, the concentration of Ca was above 3000 mg kg^{-1} and the lowest values were recorded in the year 2016. The concentrations of Mg ranged from 190-235 mg kg^{-1} throughout the experiment, and the highest values were recorded in the year 2017. The lowest value of percentage N was revealed in the first two years of study (< 0.2%). After that there was found the successive increase of % N in the soil with the peak (0.25%) in the year 2017.

The very high variability of percentage C_{org} was found throughout the study. The lowest value of % C_{org} was recorded in the year 2016 (below 2.0), whereas the highest in the year 2015. The observed significant difference in this present study was contrary to a recent one-year study in the area which reported that soil concentrations of N, P, K, Ca, and Mg increased non-significantly over time (Skrivan et al. 2015). This present study is a longer-term experiment and long-term poultry pasture has been reported as a factor that increases nutrient contents in soil and herbage (McGinley et al. 2004; Glatz et al. 2005; Hilimire et al. 2012), and this was probably the reason for the discrepancies between our result and the others.

Soil N and Mg content revealed a statistically significant increase during the grazing period of 127 days. A rise in Mg accumulation was probably caused by increase in N (Reddy et al. 2008), and increase in soil N might be attributed to high poultry litter deposition from the birds, and this finding agreed with the work of other authors who reported that fresh and/or composted poultry litter had similar N nutrient content to that of commercial fertilizer, urea (Reddy et al. 2008).

Table 1. Soil chemical properties under CG in different years and seasons investigated (Mean ± SE). 2013 Spring was the sampled result before grazing. Note: Mean values followed by the same letter (s) across columns are not significantly different (P <0.05)

	pH/$CaCl_2$	P	K	Ca	Mg	N	Corg	C/N
		mg kg^{-1}				%		
2013 Spring	6.77±0.06a	127.7±5.37a	295.3±12.79ab	3539±123a	210.9±5.30b	0.19±0.00c	2.18±0.02bc	11.11±0.17a
2013 Autumn	6.77±0.07a	101.4±8.07ab	240.5±11.30b	3248±97c	193.9±5.86b	0.18±0.00c	2.10±0.04cd	11.53±0.12a
2014 Autumn	6.44±0.07c	94.6±5.72b	298.2±10.16a	3256±66bc	211.4±5.82b	0.23±0.01b	2.32±0.03ab	9.97±0.07b
2015 Autumn	6.66±0.02ab	118.9±6.59ab	344.5±16.47a	3369±73c	214.5±4.46b	0.24±0.00b	2.43±0.05a	9.92±0.11b
2016 Autumn	6.63±0.01abc	115.2±6.91ab	292.5±13.54ab	3134±66c	205.6±4.93b	0.22±0.01b	1.97±0.04d	8.82±0.11c
2017 Autumn	6.51±0.05c	122.6±6.06a	322.3±13.45a	3530±87ab	235.8±6.36a	0.25±0.01a	2.32±0.04ab	8.97±0.32c
F-statistics	8.04	3.85	6.88	3.46	7.22	29.87	16.59	44.27
P-value	< 0.001	0.004	< 0.001	0.007	< 0.001	< 0.001	< 0.001	< 0.001

Table 2. The characteristics of the chicken grazing in the years 2013-2017

Year	Chicken genotype	Grazing period	Number of floorless portable pen	Number of chicken floorless portable pen	Chickens per m^2 Stocking rate
2013	Ross 308	1. - 18.6. (19 days)	1	1*96	8.9
2014	Hubbard JA757	14. 8. - 9. 9. (26 days)	3	1*90, 2*45	8.3 and 4.2
2015	Hubbard JA757	5. 5. - 2. 6. (28 days)	3	1*90, 2*45	8.3 and4.2
2016	Dominant	27.6.- 24.7. (27 days)	3	3*90	9.3
2017	Hubbard JA757	3.5.- 30.5. (27 days)	3	3*90	8.3

Similarly, several authors have reported cases of significantly high increased in soil N and Mg in poultry farm plots when compared with the control plots (Hilimire et al. 2012; Xu et al. 2014; Miao et al. 2015; Su et al. 2018). On the other hand, the work of O'Bryan et al. (2015) reported that no dissimilarity in soil total N content was found between the poultry grazed and the non-poultry grazed soil. Therefore, it could be concluded that the differences between farms, stocking rates and duration of study might be good reasons for the dichotomy in the N contents between poultry impacted and non-poultry soils.

Chicken grazing did not increase the soil pH in this study, instead the pH before grazing was slightly higher than after grazing. This could probably be caused by soil acidifying effects associated with organic manures especially when NH_4^+ is converted to NO_3^- and H^+. Thus, ions are released into the soil leading to decrease in pH (Sommer and Hutchings 2001). In contrast, several authors have found higher pH in poultry manure soil than in non-poultry manure soil (Berton and Mudd 2000; Su et al. 2018; Kratz et al. 2004; Hilimire et al. 2012; Kobierski et al. 2017).

Higher Ca content in the soil was expected in the sites after grazing in accordance with literatures from previous studies (Berton and Mudd 2000; Xu et al. 2014). However, Ca concentration in the soil did not increase after grazing. Differences in climate, soil, management, bird species, and feeding ration might be responsible for the disparities in results from other studies where Ca increased after grazing.

Furthermore, the study found a marginally higher concentration of P in the sites before grazing when compared with the sites after grazing. Other studies in the same issue concluded that pastured poultry plots had elevated soil P, K and total C when compared with the control plots (Hilimire et al. 2012). It seems that stocking rate and length of grazing period play important role in the increase of P.

Although the differences were not much but it is important to mention that after the first two years of experiment, the concentration of K was found to be higher in the chicken grazed sites than before grazing. This could be attributed to the fact that K takes more time to accumulate in acidic and poultry impacted soil. In support of the present result, poultry litter was also reported to have increased the soil concentration of K from 134 kg ha^{-1} to 148 kg ha^{-1} after long periods of introduction in Brazil (Pitta et al. 2002). However, higher C/N ratio was found in the initial year of the experiment, but this ratio decreased in the later years, and this could likely be attributed to the differences in biophysical factors which controlled nutrients mobilization during decomposition. This consequently had influence on carbon, nitrogen, and phosphorus dynamics. This result was consistent with other authors' report that at the beginning of decomposition, nitrogen and phosphorus tend to be immobilized in either boreal and temperate climates resulting to lower C/N ratio than in the initial ratio (Manzoni et al. 2010). Further, increase in N due to grazing activities could also be a factor responsible for the decrease in C/N ratio during the study. Some past studies have related decline in C/N with rise in N (Pérez-Suárez et al. 2014). On the contrast, soil C/N ratio was reported to have consistently increased under grazing conditions which was caused by limiting N (Pineiro et al. 2010). However, the effects of grazing on the soil C/N ratio was found to be varied based on the different soil depths in a study conducted in the steppe of China–Mongolia border (Bai et al. 2012).

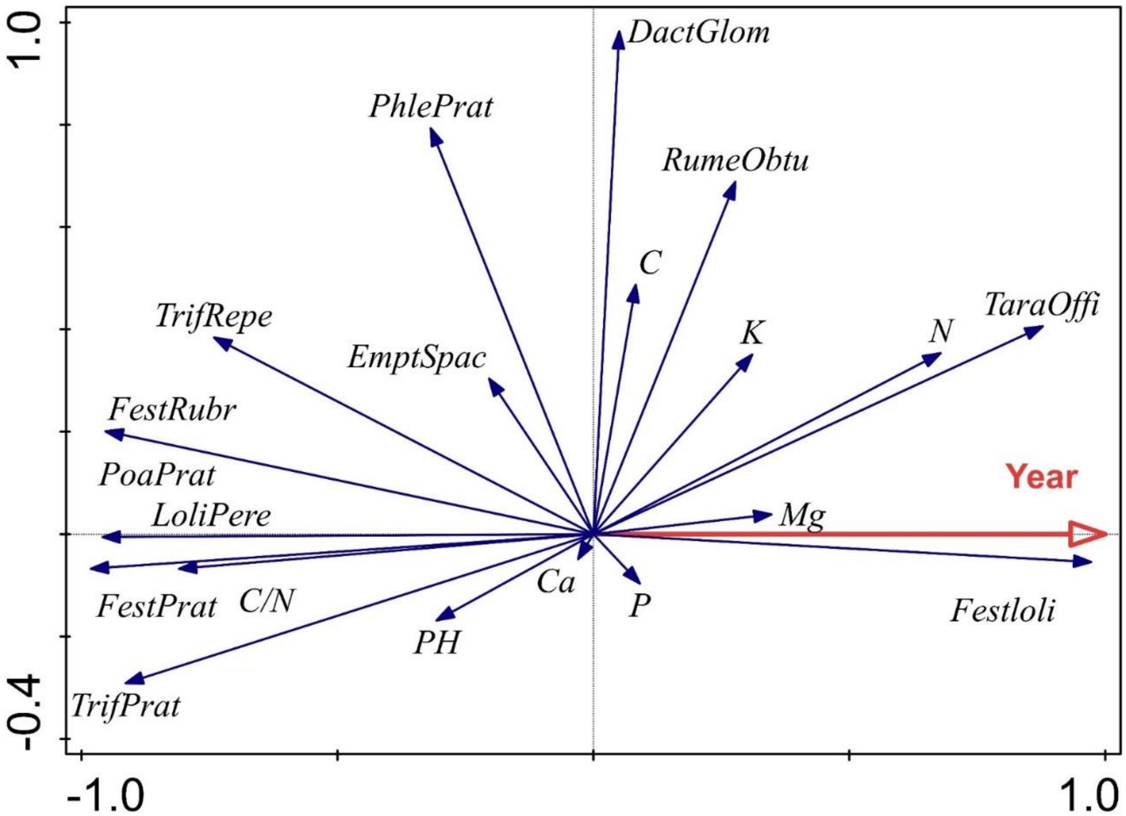

Figure 1. RDA showing the relationships between plants species and soil chemical properities in a chicken grazed pasture

Based on the RDA analysis, there were significant effect (P = 0.001, F=162) of five years of chicken grazing on soil and vegetation and this was explained by 70.4% variability (Figure 1). This indicates that CG did not only enert the dynamics in soil nutrients, but also prompted variability of the floristic composition. For example, in the year 2013, the temporary grassland consisted of three main dominants plant species: *Festulolium*, *Trifolium pratense* and *Lolium perenne* (Table 3). The CG was unfavourable for *Lolium perenne* from the second year of study. However, for *Trifolium pratense* the CG effect was already after the first year of the study.

Table 3. Botanical composition (%) in the years 2013-2017. The originaly sown species are in bold

Vascular plant species	2013	2013	2014	2015	2016	2017
Dactylis glomerata	1	0.1	2	5	0.1	0.1
Festulolium	**20**	**20**	**28**	**33**	**51**	**54**
Festuca pratensis	**5**	**3**	**2**	**1**	**1**	**0.1**
Festuca rubra	**5**	**3**	**2**	**2**	**0.1**	**0.1**
Lolium perenne	**30**	**20**	**20**	**5**	**5**	**2**
Phleum pratense	1	1	1	5	0.1	0.1
Poa pratensis	**5**	**3**	**2**	**2**	**0.1**	**0.1**
Rumex obtusifolius	1	1	1	5	2	1
Taraxacum officinale	1	1	25	25	30	33
Trifolium pratense	**20**	**30**	**2**	**1**	**0.1**	**0.1**
Trifolium repens	1	2	5	1	0.1	0.1
Other species	1	0.9	1	1	1.4	0.3
Empty spaces	9	15	9	14	9	9

On the other hand, the cover of sown *Festulolium* was favoured by CG as it successively increased from 30% to 54%. The weedy species *Taraxacum officinale* increased in cover from 1% in the 2013 to 33% in 2017. *Festulolium* and *T. officinale* were the main vascular plant species that increased their cover throughout the study (Table 3). On the other hand, all other sown plant species decreased in their cover (*F. pratensis*, *F. rubra*, *L. perenne*, *P. pratensis*, *T. pretense)*. The changes in botanical composition was caused because of two main factors: Firstly, increased N concentration in the soil and secondly grazing preferences of chicken (Miao et al. 2005; Liu et al. 2013). They prefer young plant tissue herbage with low fiber content, therefore *Festulolium* with higher fiber content started to dominate in the sward. CG is essential in the soil fertility (Zublena 1993, Foreman and Long 2013) as well as provides the opportunities to enhance the floristic composition of the grassland with more animal-preferred plant species (Liu et al. 2013).

6.3 CONCLUSION

Soil N content showed significant difference between the poultry grazed soil and the non-poultry grazed soil, and this might be explained by the differences between farms, stocking rates and duration of study. Contrary to our expectation, Ca concentration in the soil did not increase after grazing. This was probably because of environmental factors, management and species of birds used for the experiment. High C/N ratio was observed in the first year of the study which was followed by a decline ratio in the subsequent years, and the controlling effects of the biophysical components on carbon and nitrogen immobilization is possibly a good reason. Generally, our study did not find much increase in the nutrients except N, and this increase in N could pose a risk because it might lead to leaching, eutrophication and pollution of nonpoint-water and underground water due to high concentration of NO_3^-level. This might consequently be harmful to both the botanical composition and soil. The importance of CG can never be overemphasized in grassland management because it is not only a source of soil enrichment, but also helps in improving botanical composition since the birds have vegetation preference. However, CG tends to be more ecological friendly for soil when sustainably managed, but it requires longer-years of experiment for its impacts to be obviously noticed in the soil parameters.

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CHAPTER SEVEN

7.1 Discussion overview

In **case study one**, it was found that at the MSW dump sites, the soil contents of Cr and Pb in the dump sites were far above the permissible limits for agricultural soil. However, our result was also higher than the data from Taiyuan City of China (Liu et al. 2015) but in agreement with the reports from Southern Nigeria (Obiora et al. 2016), and Beijing-China (Khan et al. 2013). The concentration of Zn had a positive correlation with Cr, P and K concentrations, and a negative correlation with the concentration of N. The observed negative correlation between the concentration of Cr and N could probably be explained by inadequate N_2 - fixing plants due to the high contents of HM (Obiora et al. 2016), thus affected P and K contents too. As essential element for higher plants, Zn is phytotoxic at elevated contents and this consequently had adverse effects on the soil fertility and plant growth (Alloway et al. 1990). The concentration of Zn in this study might have increased the concentration of P and K but, availability of the later minerals for the plants' uptake could have been hindered by the high content of Cr and Pb in the soil. The plants differed in their responses to the HM. Some plants were more tolerant to the HM, thus were likely to possess higher contents of metals and still strived than other plants. Our study for example, indicated that legumes and grasses showed high concentration of Pb, shrubs were more associated with Cr, while forbs were more tolerant with Zn. The mean HM transfer factor revealed that Zn had the highest metal transfer factor from soil to plant while, shrubs and herbs showed the highest concentrations of the metals which developed an order of shrubs > herbs > legumes > grasses > forbs. *Cajanus cajan* and *Chromolaena odorata* were astounding in absorbance of HM, and they have been known as plants with potentials to tolerate HM. This might be a reason why they are commonly used as biophytoremediation by the local people (Aprill and Sim 1990; Bada and Olarinre 2012; Ismail et al. 2014).

This research work revealed that the leaves of the plants had the highest concentrations of the metals while, the roots/tubers had the lowest concentrations producing the order: leaves > shoots > roots/tubers. This might be attributed to excessive rate of transpiration by the plants to keep their water balance (Lato et al. 2012; Obiora et al. 2016; Tani and Barringston 2005). Another possible reason for the high concentrations of metals in the leafy parts of the vegetables could be explained by the particulate contaminants of plants from the dumps and/or vehicles aerosols which might be more important than the plants' uptake via their roots (Ferretti et al. 2006; Mosbaek et al. 2009; Ogunfowokan et al. 2009). Furthermore, the study found that at the control sites, species richness increased with land area, and this finding was country to the record from the dump sites.

In **case study two**, the concentration and stock of SOC and STN were compared between the grassland and neigbouring land use types. The study observed low SOC and STN status in grassland relative to the forest land. Lower SOC and STN contents in the grassland might be because of the acute use of the plants' residues for livestock fodder, fuel and other unsustainable management practices which in turn exposed the soil to severe erosion (Addis et al. 2016; Li et al. 2017; Negasa et al. 2017; Udom and Ogunwole 2015). Cultivation and grazing led to frequent harvesting and uprooting of the plants which consequently removed nutrients from the soil (Haileslassie et al. 2005) and exposed the available organic matter (OM) to soil moisture aeration and decomposing agents. This practice promoted rapid degradation and mineralization of the available OM thus, lowering the SOC and STN concentrations and stocks. Though grazing can increase SOC if managed properly (Xu et al. 2016), nevertheless vegetation types and root systems under different land uses could also influence the contents and stocks of SOC and STN (Li et al.

2017; Wang et al. 2015). For instance, tree leguminous plant species (such as *Leucaena leucocephala*, *Gliricidia sepium*, *Pentaclethra macrophylla B*.), and plant species with broad leaves, large canopies and extensive fine root systems dominated the FL contributing to high SOC and STN compared with other land uses in the study area. Additionally, grassland recorded higher bulk density (BD) because of ruminant activities which caused serious soil compaction (Liu et al. 2018), there by impairing the essential components of the soil and constraining the growth of the plant communities. Meanwhile, livestock activities can improve the fertility of the soil and increase plant species richness under proper management (Nwaogu et al. 2019), but on the other hand, the effects might be devastating to the biodiversity of the grassland in a continuously intensive grazing management.

Case study three as previously mentioned was performed in the Jizerské hory Mts. Ten years prior to the establishment of the experiment, the site was agriculturally improved by draining, then limed fertilized, and reseeded with a grass-clover mixture favorable for optimal forage yield, such as *F. pratensis*, *T. pretense, T. repens, D. glomerata*, and *P. pretense*. In the subsequent ten years after the establishment of the experiment, the meadow was cut twice each year and periodically grazed by cattle. *Arrhenatherion alliance* was a typically classified plant community in this study area (Chytrý 2007), and before the start of the study in 2000, the dominant vascular plant species were *P. pratense, F. pratensis, D. glomerata, Galium album*, and *Veronica chamaedrys*. In at least five years before the start and during the period of the experiment, there was no fertilizer applied to the meadow. The average yield of forage was about 5 t ha^{-1} of dry matter per year. The introduced treatments were: unmanaged control, two cuts per year with removal of cut biomass in June and August, mulching performed once per year in July, mulching twice per year in June and August and mulching three times per year in May, July and September. The study aimed to evaluate the effects of different management regimes (cutting, mulching and unmanaged) on vegetation characteristics of a previously improved upland meadow for 13-years. At the end of the 13 years of experiment, it was discovered that the plant species richness was positively affected by cutting and multiple mulching in relation to the unmanged. In Central Europe, longer time span of not engaging the grasslands for any agricultural purposes might lead to a rapid decline in plant species diversity. Thus, to eradicate this decrement in species diversity, at least twice mulching per year has been found the most appropriately recommended management option (Gaisler et al., 2013). Our study further observed that in repeated defoliation management, many short forbs that have prostrate, rosette or creeping growth habit (such as *T. repens*, *Taraxacum spp., P. lanceolata*) increased, whereas *T. repens* decreased. Two cuts per year enhanced the cover of *H. lanatus* while, once-mulched treatment favored *Arrhenatherum elatius*. It has been common to find *H. lanatus* dominant in fertile soils (Grime et al., 1988) but amazingly, its high existence in our experiment was observed in the two-cut treatment with the low soil nutrient status. This led to a probable conclusion that its preference for light (Grime et al. 1988) in mown meadows (Hejcman et al. 2010) preceeds its trait for fertile soil. *Festuca rubra*, though as a high plasticity plant can be seen in temperate grasslands in different management (Gaisler et al., 2013; Pavlů et al., 2007; Pavlů, Pavlů, et al., 2011) and nutrient conditions (Hejcman et al., 2014; Kidd et al.2017). It had about 5% cover in all treatments at the start of our experiment but in later years was found only in unmanaged treatment. Plant species in Central European temperate grasslands tend to have high resilience to different types of defoliation management: thus, even after eight years of the applied treatment, our findings on the tall-short species ratio showed that the obvious changes occurred only in unmanaged and in once-mulched plots.

In terms of the plants' life strategies, C strategy species dominated the study with most species found in unmanaged plots than in plots cut yearly. The proportion of C strategy species were inverse to the proportions of S and R strategy species during our experiment. The study observed that comparably with unmanaged grassland, mulching conducted once a year in improved upland meadows stimulates high weedy species and low species richness. In summary, mulching performed at least twice a year might be beneficial to grassland but could not be totally approved as a substitute to traditional two-cut management, without some decline in plant species richness.

Case study four like case study three was also performed in Jizera mountain. The major differences were that the case study four (i) had short experimental period, (ii) is dominated by *Agrostis capillaris, Festuca rubra agg., Trifolium repens and Taraxacum officinale* (Ludvíková et al. 2015; Pavlů et al. 2007), (iii) recorded pasture productivity of 2-4 t DM ha^{-1} year-1 (Pavlů et al. 2007), and (iv) was mainly focused on grazing with two paddocks in each block having two contrasting levels of grazing intensity: (a) extensive grazing (with a mean target sward surface height of greater than 10 cm); and (b) intensive grazing (with a mean target sward surface height of less than 5 cm). Additionally, three types of tall sward-height patches of (>10 cm) were identified: i) IG_TF - tall patches in IG with presence of residual spring faeces; ii) EG_TF - tall patches in EG with presence of residual spring faeces; iii) EG_T0 - tall patches in EG without presence of residual spring faeces. In respect to the IG grazing intensity, we were unable to find any presence of the tall sward-height patches without faeces. Tall patches were compared with frequently grazed patches in intensive (IG_C) and extensive (EG_C) grazing. This case study aimed at evaluating *the effects of heifers' grazing pressure on the nutrient concentrations in the soil under tall sward-height patches and in the herbage of A. capillaris grassland*. It was hypothesized that *the presence of excreta especially faeces, deposited by heifers, on tall sward-*

height patches would significantly affect the amount of nutrients in the soil and consequently in the herbage.

The result disclosed that the type of patches had no effect on the concentrations of P, K, Ca, Mg, C_{org} and N_{tot} in the soil. The quality of the nutrient content and the quantity of dung dropped by heifers on the sward surface were among other factors that possibly have influenced this result. Substantial concentrations of N, P, K and Mg in the herbage were recorded in the tall sward-height patches with faeces presence (IG_TF). Intensified over-fertilization from the heifers' excreta might likely be the best explanation/reason.

In the herbage, a decrease in nutrient concentrations (especially N and Mg) were noted in all patches under extensive grazing (EG_C, EG_TF and EG_T0). This might be attributed to the fact that herbage at early stages of maturity often possess higher nutrient contents relative to the late grazing season (Pavlů and Velich 1998; Pavlů 2015). Secondly, plants advancement in growth and maturity have been associated with a nutrient "dilution effect" (Duru and Ducrocq 1997; Pavlů 2015).The recorded non-relationship between nutrient concentrations in the soil and in the herbage observed from the regression analyses could probably be caused by plants uptake because, our data were presented in respect to amount of nutrients in the herbage per unit of area. This result agreed with the report from Dickinson and Craig (1990), that low nutrient in livestock faeces are occasionally not connected with increase in nutrients in the soil, but that the nutrients might have been instantly usurped by the plants in the dung once the nutrients were extricated/unleashed from the dung.

The study concluded that the presence of dung increased the P and K concentrations in the herbage in tall sward-height patches in intensively grazed pasture only, whereas no effect of faeces was observed in extensively grazed pasture, which could presumably be accredited to a "dilution effect".

Case study five which focused on chicken grazing was performed in Netluky village, Czech Republic between 2013-2017. This experimental site was sown by the grass/clover mixture (35 kg of seeds per ha) in early Spring in the year 2012. The mixture consisted of *Festuca pratensis, Festuca rubra, Lolium perenne, Poa pratensis, Trifolium pretense,* and the intergeneric hybrid (*c.v. Felina*). In each season, grassland was cut, and hay harvested prior to the chicken grazing performed in floorless portable pen. The genotypes of the chickens used were Ross 308, Hubbard JA757, and Dominant. This study aimed at *determining whether soil from longer-term CG enhanced soil nutrient concentration in comparison with soil status before grazing*. It is hypothesized that *CG caused increased soil N while, preferential grazing by the chickens led to changes in botanical composition*. The result indicated significant differences in all the soil chemical properties monitored though, the trend was not obviously defined across the years. The observed significant difference in this present study was inconsistent with a recent one-year study in the area which reported that soil concentrations of N, P, K, Ca, and Mg increased non-significantly over time (Skrivan et al. 2015). Differences in the study duration is possibly the reason for the disparities in our study and others. For instance, some authors have reported that long-term poultry-pasture experiment increases nutrient contents in soil and herbage (McGinley et al. 2004, Glatz et al. 2005, Hilimire et al. 2012). Soil N and Mg content revealed a statistically significant increase during the grazing period of 127 days. A rise in Mg accumulation was probably caused by increase in N (Reddy et al. 2008). On the contrary, O'Bryan et al. (2015) observed no dissimilarity in soil total N content between the poultry grazed and the non-poultry grazed soil. Thus, differences between farms, stocking rates and duration of study were the likely explanation for the distinction in the N contents between poultry impacted and non-poultry grasslands. Based on previous literature on this issue, it was not amazing to discover a higher Ca concentration in the soil after grazing (Berton and Mudd 2000, Xu et al. 2014).

In terms of the floristic composition, chicken grazing was suitable for the cover of *Festulolium* (rose from 30% to 54%) and the weedy species *Taraxacum officinale (*increased from 1% to 33%) in 2013 to 2017 when compared with the two other species in the study area. In contrast, all other sown plant species (such as *F. pratensis, F. rubra, L. perenne, P. pratensis, T. pretense*) declined in their cover. Two key factors might be held accountable for the changes in botanical composition. These factors were: elevated N concentration in the soil and grazing preferences of by the birds (Miao et al. 2005, Liu et al. 2013). The chickens prefer young plant tissue forage with low fiber content, hence *Festulolium* with higher fiber content increased in the sward. Besides being identified as important for soil quality enhancement (Foreman and Long 2013), chichen grazing supports grassland management by helping the farmers introduce divers species with higher palatability (Liu et al. 2013).

7.2 Principal conclusions and summary

The primary goal of this work is centered on *appraising the dynamics of elements in the soil and plants in grasslands under different management (such as gazing, cutting, mulching, and waste disposal).*

This key goal is realized with the following five structured hypotheses which were consistent with individual case study (e.g., case study 1-5 as presented in chater 2-6).

The hypotheses were that:

1. in addition to the significant effects in the soil and in the plant chemical properties, the MSW dumps affect the species composition and richness.

2. the concentrations and stocks of SOC and STN are significantly affected by slope position, soil bulk density, and depth.

3. different meadow management methods (mulching, cutting and abandonment) affect long-term successional changes in plant species composition, main plant functional groups, and species richness.

4. the presence of faeces, deposited by heifers, on tall sward-height patches would significantly affect the amount of nutrients in the soil and consequently in the herbage of *A. capillaris* grassland.

5. chicken grazing (CG) caused increased soil N while, preferential grazing by the chickens led to changes in botanical composition.

The previous chapters might have been deemed to be rather voluminous with comprehensively large information. On this note, and as a necessity, it was conceived to elucidate at a glance some important accomplishments regarding the study goal and objectives by using the following paragraphs.

Case study 1: Variations of elements in soil and plants due to municipal solid waste dumps in grasslands

Long-term indiscriminately created municipal solid wastes (MSW) dumps are openly seen on the grasslands along the main roads linking the sub-urbs and the major cities in Nigeria. Despicablly, the wastes have been nonchalantly or ignorantly abandoned by the people and government while, the heaps increase daily in most cases with several impacts on the soil and flora (such as

Andropogon gayanus, Brachiaria decumbens, Cynodon dactylon and Cajanus cajan). This prompted our study which aimed at investigating the responses of the soil and plant chemical properties to heavy metals (HM) induced by MSW. It was hypothesized that the metals from the wastes affected the soil and plants at the dump sites relative to the non-dump (control) sites. Findings from the analyses confirmed that the effects of HM were significant and high at the dump sites with concentrations far above the EU, and Canadian environmental quality permissible limits for agricultural soils and vegetation. The study further observed that (i) contrary to the dump sites, a significant relationship ($R^2 = 0.70$; $p < 0.001$) was found between the number of plant species and area at control sites, (ii) shrubs and herbs were more tolerance to higher contents of HM when compared with grasses. At this juncture, it could be concluded that the aim and hypothesis of the case study 1 were justifiably achieved in line with the main goal of this work.

Case study 2: Evaluating the differences in SOC and STN status in grassland and neigbouring land use.

Agricultural-rich Imo watershed which is in south-eastern Nigeria was the study site for this case study 2. It has a total area of 4321.4 km^2 with grasslands covering more than 54% of the entire landmass. The dominant plant species were *Andropogon gayanus, Brachiaria decumbens, Cenchrus ciliaris, Cynodon dactylon, Pennisetum pedicellatum, Panicum maximum, Panicum purpureum.* Since several decades ago, the area has been used for agicultural purposes without fertilization. Lately, soil degradation effect has begun to be noticed by the smallholder farmers as their yields are on the decline. Poor farming system and climate change tend to be the major reasons. Dearth of soil data in the watershed motivated the need to examine the dynamics of SOC and STN conditions among other soil properties in this area. The objective was to *quantify the dynamics in soil properties under different land use in Imo watershed where there is no knowledge about the effects of land use on SOC and STN pool.* We hypothesized that *the concentrations and* *stocks of SOC and STN are significantly affected by slope position, soil bulk density, and depth*. By employing ArcGIS 10.1 and FAO land use classification system, six land use (grassland, arable land, forest land, shrubland hills, urban built-up green, and mangrove wetland) were identified. The study observed low SOC and STN status in grassland relative to the forest land. And this low SOC and STN contents in the grassland is likely caused by profound use of the plants' residues for livestock fodder, fuel and other unsustainable management practices which in turn exposed the soil to serious erosion and leaching. The result further revealed that (i) topsoil layer (0-30cm) contributed to more than 90% of the total soil nutrients, (ii) land use significantly affected SOC content, STN content, and bulk density, (iii) slope position and soil depth also significantly affected SOC and STN contents. The study was a good move to provide land managers and farmers the information to enrich soil quality, conserve C and N stocks for food security, sustainable environment and climate change mitigation. In sum, the aim of this case study 2 was therefore met in accordance with the main goal of the study.

Case study 3: Plant species composition responses to long-term effects of mulching, traditional cutting and no management of improved upland grassland.

By addressing the question: '*how do different meadow management methods (mulching, cutting and abandonment) affect long-term successional changes in plant species composition, the main plant functional groups, and species richness?*', the objective of the study was defined. As to proffer solution to the challenges of inadequate livestocks for the management of both semi-natural grasslands and previously intensified upland grasslands, mulching frequencies and traditional cut management were introduced as experimental treatments. After long term experiment, it was observed that (i) traditional management of two-cuts with biomass removal was the most suitable method for maintaining plant species richness and diversity, (ii) mulching (especially once per year) recorded high weeds and low species richness like umanged treatment, hence is a preferred option for the abatement of shrubs and trees encroachment. On the other hand, repeated mulching, though better than once-mulching in maintain species richness, yet it cannot be completely used in place of traditional two-cut management for improving upland meadows without reducing plant species richness and diversity. On this note, it is reasonable to agree that the aim of this case study 3 has been practically actualized in line with the main goal of the thesis.

Case study 4: Effects of grazing and dung on nutrient level in herbage and soil

By answering the following questions: *(i) what is the effect of the presence of faeces on nutrient concentrations of soil beneath tall sward-height patches under intensive and extensive grazing management? (ii) does the presence of faeces on the surface beneath tall sward-height patches affect dry standing biomass yield, dry matter (DM) content, dead biomass, and nutrient concentrations in the herbage? and (iii) is there any relationship between soil nutrient concentrations and herbage nutrient concentrations under the tall sward-height patches?*

The objective and hypothesis of this case study were satisfactorily addressed. For example, the result indicated that (a) different types of patch had no significant effect on soil available nutrients, (b) herbage from the different types of patches differed significantly in concentrations of N, P, K, Ca and Mg, (c) the highest nutrient concentrations for P and K in the dry matter (DM) herbage were in patches with faeces under intensive grazing. Conclusion was drwn that the presence of faeces hightened the P and K concentrations in the herbage in tall sward-height patches in intensively grazed pasture only, whilst no effect of faeces was observed in extensively grazed pasture which is likely due to a “dilution effect”. Thus, signifying that plants uptake of the minerals from heifer dungs was probably the reason for low soil nutrient.

Case study 5: Chicken grazing (CG) effects on soil and plants, Czech Republic

By focusing on the effects of CG on soil nutrient concentration and floristic composition under five years field study in sown grassland, we hypothesized that 'though, chicken grazing caused increase in soil N yet, their preference in forage grazing induced changes in botanical composition'. The result revealed that N and plant available Mg were the only nutrients that got elevated during the study under CG. However, the study recorded a decline in the percentage of sown high productive species, in excemption of *Festulolium* and increasing cover of weedy *Taraxacum officinale*. Though, CG seems to be suitable management for the maintenance of soil fertility status, but it could be detrimental over long time because of increase in N and change in structure of sward. On these records, it was strongly believed that the aim of this case study 5 was harmoniously fulfilled in accord with the main goal of this work.

7.3 Recommendations from the thesis

Apart from Cr, Pb and Zn, further study on the effects of other HM on soil and plant is recommended in the area. The government and stakeholders in nature conservation should spur to action by enacting laws that instigate stringent punishment on any indiscriminant waste disposer. Moreover, the introduction of biological methods such as recycling and bio-phytoremediation processes which have been proved to be sustainable in ameliorating the effects of HM should be adopted.

Enhancing the SOC and STN status means improving the soil fertility. Therefore, sustainable farming systems such as conservative agriculture is recommended since there is high cost and inaccessibility of mineral fertilizer in the area. Further studies on the cycling and variability of other essential nutrients are necessary as to consolidate the findings of this present research and restore the soil quality of the grasslands in the watershed.

However, Chicken grazing has been identified as a profitable method for soil improvement, but long-term experiment is necessary to fully ascertain and complement the findings of this study.

7. 4 References

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CHAPTER EIGHT

8.1 List of Author's Publications

Publications (in WOS and Scopus)

Chukwudi Nwaogu, Vilém Pechanec, Vit Vozenilek. 2019. Responses of soil and plants to spatio-temporal changes in landscape under different land-use in Imo watershed. Archives of Agronomy and Soil Sciences. http://dx.doi.org/10.1080/03650340.2019.1566714

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8.2 List of figures

As in chapter two (case study 1)

As in chapter three (case study 2)

As in chapter four (case study 3)

8.3 List of Tables

As in chapter three (case study 2)

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8.4. List of Appendices / Supporting information