Effect of plant and animal functional traits on nutrient cycling in low-input pastures

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„In the flinty light, it's midnight, and stars collide
Shadows run, in full flight, to run, seek and hide
I'm still not sure what part I play,
in this shadow play, this shadow play.“
Rory Gallagher, 1978

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General Introduction

In Europe 30 % of the agriculturally utilized area is permanent grassland (Smit *et al.*, 2008). Semi-natural temperate grasslands are considered as a relevant resource of biodiversity in agriculture (Isselstein *et al.*, 2005). Grazing has been recognized as the most efficient way to maintain and utilize these low-input and semi-natural grasslands (Rook *et al.*, 2004; Wrage *et al.*, 2011).

In grazed low-input pastures, which are mostly nitrogen (N)-limited, the absence of fertilizer use induces that nutrient cycling is primarily mediated by forage uptake and excrement deposition of grazing animals (Rotz *et al.*, 2005). In this process specific characteristics of animal (e.g. grazing selectivity, size of excrement patches) and plant (e.g. height, specific leaf area index), so-called 'functional traits', play an important role for the cycling of nutrients within the system. Plant functional traits determine the plant response to available nutrients (Pastor & Bridgham, 1999; Cingolani *et al.*, 2005) and attractiveness to grazers (Bardgett & Wardle, 2003). Animal functional traits influence the forage uptake (Lloyd *et al.*, 2010), size and pattern of nutrient return (Williams & Haynes, 1994).

Nutrient cycling through herbivores always leads to spatial separation and concentration of nutrients (Auerswald *et al.*, 2010). Due to the low retention of nutrients of the ingested herbage in animal tissue, a high proportion of nutrients is recycled rather than removed from the grazing system (Rotz *et al.*, 2005). Herbivores may excrete between 75 to 90 % of N (Ball *et al.*, 1979) and over 70 % of phosphorus (P) consumed (Watson & Foy, 2001). They quantitatively separate nutrients between urine and dung, whereby most of the P taken up is excreted in dung (over 70%), potassium (K) predominantly in urine (up to 90%) and N in differing proportions in both urine (45-80%) and dung (20-55%) (Whitehead, 2000). Under low-input conditions the N limitation may lead to a lower proportion of N being cycled in urine (Whitehead, 1995).

Urine and dung patches differ in their nutrient availability directly following the deposition (Haynes & Williams, 1993; White-Leech *et al.*, 2013a; Selbie *et al.*, 2015). N in urine is primarily present in the form of urea, which is rapidly hydrolized and therefore plant-available shortly after deposition (Williams & Haynes, 1994), while a high proportion N in dung is organically bound (White-Leech *et al.*, 2013a) and mineralization can take up to two years

(Saarijärvi & Virkajärvi, 2009). Also K is readily available in urine, as it is water-soluble and in ionic form (Haynes & Williams, 1993). Degradation of dung patches increases the P and K availability (Aarons *et al.*, 2004).

The substantial differences between animal species in terms of size and redistribution of excrements over the pasture directly affect the nutrient cycling (Williams & Haynes, 1994). Sheep urinate 18-20 times per day with a volume of 0.1-0.2 l per urination, while cattle urinate less frequently (10-12 times) with a higher volume (1.6-2.2 l). The area covered by a single urination is 3.5 times greater for cattle than for sheep urine patches. Cattle may excrete 10-16 dung patches per day with a weight of 1.4-2.7 kg each, while sheep defecate more frequently (19-26 times) with a lower weight per defecation (0.03-0.17 kg) (Haynes & Williams, 1993). The urine and dung patches represent a concentration of nutrients taken up from large grazing areas into the small areas covered by excrement (Afzal & Adams, 1992). Excrements of grazing herbivores are considered as ‘hot spots’ of intensive nutrient cycling in these systems (Haynes & Williams, 1993).

Apart from the animal functional traits, the grazing system is an important factor determining the distribution of excrements within the pasture (Dubeux *et al.*, 2009; Auerswald *et al.*, 2010). In continuously stocked pastures the excrements are locally concentrated near sheds and watering areas (Dubeux *et al.*, 2006; Dubeux *et al.*, 2009) and temporal evenly distributed (Dubeux *et al.*, 2009). In rotationally grazed pastures, however, the pasture subdivision is suggested to limit this concentration and consequently redistribute excrements more evenly (Williams & Haynes, 1990; Sigua *et al.*, 2010). There is only little information on the pasture vegetation response to excrement deposition at different excrement deposition times (Bélanger *et al.*, 2015).

Urine and dung were reported to affect both plant production and nutrient concentration following its deposition (Saarijärvi & Virkajärvi, 2009; Moir *et al.*, 2016). Urine patches can affect plant biomass production and nutrient concentration within a distance of 15-20 cm from the patch (Decau *et al.*, 2003; Saarijärvi & Virkajärvi, 2009; White-Leech *et al.*, 2013a, b). In contrast to this, dung patches initially decrease biomass productivity at the surrounding area and over a long term increase biomass production following its deposition (MacDiarmid & Watkin, 1971; Williams & Haynes, 1995). Most studies were performed using cattle excrements, whereas only some information on the effect of sheep urine (Marsden *et al.*, 2016) and sheep dung patches on pasture vegetation is available (Williams & Haynes, 1995; Ma *et* *al.*, 2007). Moreover, recent studies predominately focused on plant biomass N response to excrement patches, thus there is only little information on P and K in plant biomass (Aarons *et al.*, 2009).

Most studies were performed on intensively managed and fertilized pastures (Saarijärvi & Virkajärvi, 2009; White-Leech *et al.*, 2013a), for which the additional nutrient supply with excrements is of minor importance for production of pasture vegetation. In low-input grazing systems the excrements markedly change the soil nutrient status at the patches (Aarons *et al.*, 2009) and consequently affect the plant biomass nutrient concentration and production. Up to now only few studies investigated low-input pastures (Ma *et al.*, 2007).

Diverse systems received less attention in recent studies. The sward botanical composition influences the response to nutrients. In species-rich, diverse pastures, the proportion of legumes and the amount of biologically fixed nitrogen can be decreased by nutrient addition through excrements, which offsets the effect of excrement-N input (Vinther, 1998), depending on clover content of the sward and time of excrement application (Ball *et al.*, 1979; Ledgard *et al.*, 1982). On species-poor swards the nutrient application leads to an increased biomass production (White-Leech *et al.*, 2013a, b). Up to now there is no direct comparison of the response of grass-dominated and diverse swards to excrements. In low-input pastures, which are rather diverse (Rook *et al.*, 2004), this comparison would give more information on nutrient cycling in the system.

The excrement patches themselves also affect the plant-to-animal interaction in terms of local biomass uptake. Herbage growing in close vicinity to dung patches is avoided by grazing animals (Smith *et al.*, 2009; Gillet *et al.*, 2010). Avoidance of dung patches is a strategy of grazers to avoid parasite contamination (Smith *et al.*, 2009). Pasture vegetation at urine patches may be preferentially grazed within the next grazing period (Jaramillo & Detling, 1992), possibly due to increased nutrient concentration in the biomass. Differences between cattle and sheep in grazing selectivity at the excrement patches have rarely been studied (Forbes & Hodgson, 1985). Results of Hutchings *et al.* (1998) show increasing biting depths of sheep near their dung patches with increasing age of the patches. Most studies on urine and dung effects on both vegetation production and grazing animal response have so far used simulated excrement patches, neglecting the conditions of a real grazing system. Under real grazing conditions, the response of pasture vegetation to excrements (production and nutrient-uptake response) is a complex process depending on defoliation frequency, defoliation severity and time of excrement deposition. Animal excrement deposition, as well as plant and animal response to excrement patches are therefore influenced and controlled by the grazing system. There is in particular a lack of information on animal species effect on plant functional group response to excrements and grazing response to excrement patches in dependence on plant functional groups present. So far the nutrient cycling from plant-to-animal, animal-to-soil and soil-to plant were individually investigated, thus a total view of animal-to-soil-to-plant in a grazing system is missing in current studies.

Therefore a rotational grazing system with cattle and sheep grazing on either grass-dominated or diverse swards was used, to analyse the influence of grazing animal species and sward botanical composition on herbivory effects on nutrient cycling (Chapter I). Based on these results a simulated grazing system was established, using excrements derived from the rotational grazing system, to further investigate the effect of nutrient separation in urine and dung on soil nutrient status and plant biomass (Chapter II). However, under realistic grazing conditions the nutrient concentration in excrements is variable (Haynes & Williams, 1993), which is why it was additionally investigated, to what extent the plant biomass nutrient concentration responds to varying nutrient application rates with excrements (Chapter III).

The underlying questions of this work were:

- ➔ What is the effect of animal- and sward-specific responses to excrements on nutrient cycling within the stocking period subsequent to different excrement deposition times? (CHAPTER I)
- ➔ What is the medium-term effect of nutrient separation in urine and dung on plant biomass production and soil nutrient status (CHAPTER II)
- ➔ To what extent does the plant biomass nutrient concentration respond to varying excrement nutrient application rates present in a real grazing system? (CHAPTER III)

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Chapter I:

Herbage biomass and uptake under low-input grazing as affected by cattle and sheep excrement patches

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Abstract

In low-input grazing systems excrement patches are the main nutrient input. They influence both forage production and intake of grazing animals. Our goals were to determine (1) whether seasons of differing weather conditions and swards of differing botanical composition influence the excrement effect on plant biomass in grazed pasture and (2) if animal species differ in their forage intake at excrement patches. We measured the plant biomass and forage intake responses to dung and urine patches of cattle and sheep in rotationally grazed low-input pastures with either grass-dominated or diverse swards in the stocking periods following excrement deposition in spring and autumn. At urine plots the plant biomass production was 14.7 % higher than at the corresponding control plots, accompanied by a 19 and 17 % higher biomass nitrogen and potassium concentration, respectively. The effect of excrements on plant biomass production, nutrient parameters and animal forage intake was not affected by animal species, sward type or stocking period. Small-scale sward height measurements showed that cattle avoided vegetation close to dung patches in both stocking periods whereas sheep did so only following the excrement deposition in spring and that cattle preferentially grazed at urine plots on grass-dominated swards. The effect of excrement patches on vegetation biomass parameters was small, which masked potential influences by animal species, sward type and excrement deposition time.

Keywords: Nutrient cycling; Grazing selection; Forage intake; Diverse grassland; Excreta; Patch

Introduction

Grazing animals play an important role in the matter and nutrient cycle of pasture land, as most of the mineral nutrients consumed are returned to the pasture via dung and urine (Rotz *et al.* 2005). Urine and dung patches are considered as `hot spots` of intensive nutrient cycling in pastures, as ruminants predominantly excrete phosphorus (P) in dung, potassium (K) in urine and nitrogen (N) in varying proportions in both urine and dung (Haynes & Williams, 1993). Fertilized pastures, where the contribution of urine and dung to biomass productivity is less important, have been intensively investigated (Moir *et al.*, 2013; Saarijärvi & Virkajärvi, 2009; White-Leech *et al.*, 2013a, b). Unfertilized low-input grazing systems, where excrement patches are the most important nutrient source for the pasture vegetation, have hardly been studied (Ma *et al.*, 2007).

In contrast to dung, N in urine is present predominantly in rapidly hydrolized urea, which makes it plant-available shortly after deposition (Haynes & Williams, 1993; White-Leech *et al.*, 2013a). Consequently, time of excrement deposition could be crucial for plant utilization of the supplied nutrients (Moir *et al.*, 2016). A deposition of highly plant-available N in phases of low nutrient demand, like over winter, may result in high nutrient losses (Saarijärvi & Virkajärvi, 2009; Selbie *et al.*, 2015). A direct comparison of different deposition times of urine and dung patches on plant biomass of grazed pastures has only rarely been made (Bélanger *et al.*, 2015).

Apart from management, the botanical composition of the pasture is an important factor influencing the plant response to additional nutrient supply through excrement. Most studies have been performed on grass-dominated pastures, where the high N input from excrement leads to an increase in plant biomass productivity and N concentration (White-Leech *et al.*, 2013a, b). In legume-rich pastures N input from excrement was shown to lead to a substitution of the fixed N and to decrease the legume dominance (Ledgard, 2001). So far, a direct comparison of excrement effects on grass-dominated and diverse swards, with their difference in N availability and demand, is missing.

Grazed pasture systems are characterised by a spatial variability in nutrient return in excrement patches, which leads to a spatial concentration, separation and redistribution of nutrients (Auerswald *et al.*, 2010; Haynes & Williams, 1993). Here the stocking rate is an important factor determining the excrement density in the paddock (Dennis *et al.*, 2011). This factor was addressed by studies of the impact of animal species on nutrient cycling (Ma *et al.*, 2007; White-Leech *et al.*, 2013b; Williams & Haynes, 1994). Especially at the sub-meter scale, the small excrement patches of smaller herbivores lead to a more fine-scale distribution of nutrients returned in comparison to larger herbivores. The effect of animal size on nutrient cycling has hardly been studied (Bakker *et al.*, 2004; Williams & Haynes, 1994). Cattle and sheep represent animal species of different excrement size and distribution (Haynes & Williams, 1993). While urine patches of cattle and sheep can significantly increase plant biomass productivity and biomass N uptake (Decau *et al.*, 2003; Marsden *et al.*, 2016; Saarijärvi & Virkajärvi, 2009; White-Leech *et al.*, 2013a), cattle dung patches were shown to initially have a direct adverse effect on vegetation in the immediately affected area (White-Leech *et al.*, 2013a; Williams & Haynes, 1995). Information on the effect of sheep dung patches on plant biomass productivity is scarce (Ma *et al.*, 2007; Williams & Haynes, 1995), as is information on the effect of excrement on P and K concentration in the plant biomass. The mineral content of herbage is an important indicator of the nutrient supply to the plants and it contributes to the herbage nutritive value for grazers (Whitehead, 2000).

Excrement patches also have a direct effect on the local plant biomass intake by grazers. Herbage growing around dung patches is avoided by grazing herbivores (Gillet *et al.*, 2010; Smith *et al.*, 2009). In contrast, an increased plant biomass production, due to the high nitrate concentration at urine patches (Moir *et al.*, 2012), may result in a preferred grazing of these areas within the following stocking period (Day & Detling, 1990). To quantify the forage intake under grazing conditions, especially at dung patches, a small-scale resolution has to be chosen. The fine-scale height measurement (Stewart *et al.*, 2001) of vegetation surrounding the excrement patches allows a precise assessment of excrement patch effects on animal grazing selectivity.

For these reasons, a grazing experiment on rotationally grazed pastures was performed using cattle and sheep as grazer species and grass-dominated and diverse swards to represent different botanical diversity of the pasture. Urine, dung and control (no excrement) patches were marked in situ in every paddock in spring and autumn of 2014 to determine the effect of excrement patches on herbage biomass production, nutrient concentration, plant nutrient uptake and forage intake by the grazers during the following stocking period. The underlying hypotheses of this study were (1) that urine and dung would increase plant biomass production and nutrient concentration to a different extent and with difference in the reaction of diverse and grass-dominated swards and, (2) that grazing animals reject dung patches and preferentially graze at urine patches, but that cattle and sheep differ in their grazing response to excrement patches.

Materials and Methods

Setup of the grazing experiment

The experimental site, an area of 6 ha, is a long-term permanent grassland in its initial condition corresponding to a moderately species rich *Lolio-Cynosuretum*. It is located in the Solling Uplands, Germany (51°46'47 N, 9°42'11 E); the altitude of the total area ranges from 184 to 209 m above sea level. The soil type is a Vertic Cambisol (World Reference Base of Soils), the texture is clayey/silty loam. Initial topsoil (0-10 cm) mean available nutrient concentrations in 2007 were P: 79, K: 174, Mg: 358 mg kg^{-1}, mean pH was 6.8 (extraction: calcium acetate lactate for P and K, $CaCl_2$ for Mg and pH; Seither *et al.*, 2014). Topsoil total N and total C were 3 and 33 g kg^{-1}, respectively (both in dry soil). The soil analyses indicated a sufficient supply with P and K (Janssens *et al.*, 1998), whereas a positive plant yield response to N fertilization found by Seither et al. (2014) indicated N limitation. The average temperature was 8.2 °C (1961-1990, Deutscher Wetterdienst, DWD, Location: Lutterbeck - 10 km from the experimental site at the same elevation). The average annual precipitation was 879 mm (1961-1990, Deutscher Wetterdienst, DWD, Location: Dassel - 3 km from the experimental site). Weather data during the experimental period is shown in Online Resource 1.

The experiment in its current form was established in 2012. In this experiment, two paddock-scale factors were tested: botanical diversity of the pasture swards and the grazing animal species. The initial diversity of the sward was manipulated in 2006 and 2009 for a prior experiment with mixed grazing of cattle and sheep that ran from 2007-2011 (for further details see (Seither *et al.*, 2014)). Following that experiment, the sward composition was again manipulated in 2012 by the use of herbicide against dicotyledonous plants (active components Fluroxypyr/ Triclopyr), resulting in a low-diversity grass-dominated sward (‘grass sward’) compared to the untreated ‘diverse sward’ (referred to as ‘sward types’ below). Before the start of the experiment the average contribution of plant functional groups to biomass were visually estimated as 93 % grasses and 7 % herbs at grass-dominated swards, with *Dactylis glomerata*, *Poa trivialis, Poa pratensis, Lolium perenne* and *Festuca pratensis* as the main species representing >2/3 of herbage dry mass. On diverse swards the average contribution of plant functional groups were 66 % grasses, 31 % herbs and 2 % legumes, the main species being *Dactylis glomerata, Poa trivialis, Poa pratensis, Taraxacum officinale* and *Lolium perenne* representing >2/3 of herbage dry mass. Other species present can be found in Online Resource 2. Grazing animal species were cattle (adult, non-lactating suckler cows of the breed German Simmental), and sheep (adult, non-lactating Blackheaded sheep ewes). Animals were assigned to 0.5 ha paddocks. The stocking system was rotational stocking with three stocking periods per year. In each stocking period, the three experimental blocks (A, B, C) were grazed consecutively. The stocking density was based on the estimated daily animal dry matter intake, which is defined as the intake capacity (Jouven *et al.*, 2008), to achieve a similar forage intake at each paddock for both animal species. The intake capacity is calculated from the metabolic bodyweight and condition of the animals. The number of sheep per paddock was chosen based on the intake capacity of cattle. The resulting stocking density per 0.5 ha paddock was 5 cattle, or 30 sheep, respectively. The stocking period lasted from early May to mid-October with a break of about 10 weeks for animal mating between stocking periods two and three (Online Resource 3).

Marking of excrement and control plots

To assess different scenarios of plant and animal response, effects of animal excrement were observed over two periods that differed in weather conditions and in the resting interval between stocking periods. The course of the experiment is shown in Table 1. The measurements followed a triplet design consisting of control, dung and urine plot. In each paddock, three urine patches were marked by direct animal observation at the end of the respective stocking period. In close vicinity to a marked urine patch, one dung and two control plots with the same botanical composition as on the urine patch were chosen. For sheep dung patches, visually recognisable droppings of approximately 100 g were chosen. If there was a visible influence of excrement on a marked control plot in the following stocking period, this control was discarded. Otherwise, one of the two controls was randomly chosen for further measurements. This resulted in a total of 108 sampled plots for the summer and 108 sampled plots for the winter observation period.

These plots were fenced in a way that grazing within the fenced areas was possible but the contamination by excrement was prevented (Hirata *et al.*, 1988). The fences consisted of four plastic poles connected with two wires in different heights. The resulting plot had an area of 1*1 m^2 for cattle and 0.5*0.5 m^2 for sheep, respectively (Online Resource 4). The plot size for cattle was chosen according to Hirata *et al.* (1988), with a ratio of 3.5 between the urine patch size (Haynes & Williams, 1993) and the rest of the plot. The same ratio was used to determine the plot size based on the sheep urine patches, so that the results would be comparable between animal species.

Table 1 Dates for vegetation height measurement and vegetation biomass sampling at observation plots for the excrement deposition times

Observation period	Marking of excrement and control plots	Vegetation height measurement and plant biomass sampling
Summer	08/05-16/06/2014	17/06-11/07/2014
Winter	17/09/2014-27/10/2014	11/05-09/06/2015

Biomass sampling

Right before the stocking period that followed the selection of the excrement and control plots, one half of each plot was harvested at 2 cm height using electric scissors. The biomass of the other half was harvested after the stocking period. This led to the following target variables: Biomass production following excrement deposition (cut before animal re-grazing), forage residue (cut following animal re-grazing) and apparent forage intake (difference between the two cuts). The biomass was subsequently dried to constant weight for 48 hours at 60 °C. After determining the dry matter (DM) weight, the material was ground to 1-mm. The P and K concentrations of the plant biomass were determined after digestion with aqua regia using an Inductively Coupled Plasma 6300 DUO ICP OMS (Thermo Fisher Corporation, Waltham). The analysis of N in plant biomass used the Dumas combustion method with a Variomax CN (Elementar Analysesysteme GmbH, Hanau). Plant nutrient uptake of aboveground biomass was calculated as the product of nutrient concentration and DM yield.

Vegetation height measurement

The target variable of the vegetation height measurement was the stubble height. Vegetation height was measured in 5 cm intervals along previously defined transects (height = height of the first plant part that touches a measuring surface of 6 cm^2, as implemented by the sward stick; Stewart *et al.*, 2001). Based on the excrement plots the height was measured in two directions at nine points each for cattle and at six points each for sheep, either starting in the centre of the plot (urine and control plots) or at the edge of the dung patches (Online Resource 4). The mean value of the height measurement of both transects with the same distance from the start of transect was used for further calculations. Stubble height was measured following the stocking period (measurement following animal re-grazing).

Stubble height gives a two-dimensional picture of the effect of excrement patches on the vegetation height as affected by the distance from the excrement itself. The absence of a distance effect on the target variable would indicate either a uniform reaction or no reaction to the excrement patches. An increasing vegetation height with increasing distance from the excrement would indicate a preference of the grazing animal, whereas a decreasing vegetation height with increasing distance from the excrement patch would indicate avoidance by the grazing animal. To account for variation of vegetation height that was independent of the deposition of excrement, the vegetation height of the corresponding control plots was subtracted from the vegetation height of each urine and dung plot at each distance.

Statistical analyses

The statistical analysis was carried out using the program R version 3.2.0 (R Core Team 2015). Influence of the four experimental factors plot type, observation period, sward type and animal species on the response variables were examined using linear mixed effects (LME) models and the package nlme (Pinheiro *et al.*, 2015). LME models for plant biomass production, nutrient concentration and plant nutrient uptake had the fixed effects patch type, observation period, sward type and animal species, as well as their interactions, and replicate blocks with nested paddock and triplet locations of patch types as the random effects. For the statistical analysis of the vegetation height, each excrement type was tested separately for the two animal species.

Initial LME models had the distance from excrement patch, sward type and observation period as fixed effects and replicate block as the random effect. All residuals were checked for normal distribution and homogeneity of variance. Because of heterogeneous variances, a boxcox function was run for plant biomass production and forage residue in order to determine the appropriate transformation using the MASS package (Venables & Ripley, 2002). Initial LME models were simplified by removing non-significant factors or interactions, if this led to a lower value of the second-order Akaike Information Criterion (AICc). The pair-wise comparison of means in the case of significant fixed effects was performed using the LSD test as implemented in the package lsmeans (Lenth & Hervé, 2015).

Results

Plant biomass

The statistical analysis showed a significant effect of plot type on plant biomass production (Fig. 1a). Harvested biomass was increased by 14.7% at the urine plots, as compared to the corresponding control plots. The plant biomass production was neither affected by animal species, nor by observation period or sward type (Table 2).

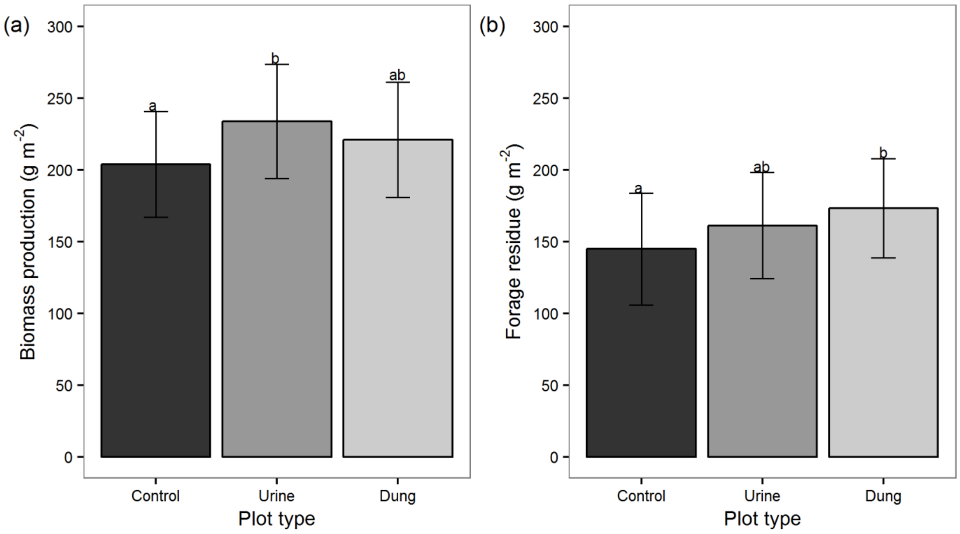

Figure 1 Mean (± standard error, *n* = 72) plant biomass production (a) and forage residue (b) of different plot types, averaged across grazing animal species, observation periods and sward types. Means with different letters indicate significant differences between patch types (linear mixed effect models with Tukey LSD test at $P<0.05$).

Table 2 Effects of plot type (urine patch, dung patch, control) on the vegetation dry matter biomass production, apparent animal forage uptake and forage residue following the stocking period, as affected by grazing animal species (cattle or sheep), sward type (grass-dominated or diverse) and observation period (summer or winter); values represent the results of the linear mixed effects models (F and *P*); main effects and interactions for which no values are presented were not included in the simplified final linear mixed effects model.

Effect	Biomass production		Apparent forage uptake		Forage residue	
	F	*P*	*F*	*P*	*F*	*P*
Plot type (P)	3.74	**0.0255**			4.62	**0.011**
Sward (S)			4.73	0.061	1.41	0.236
Animal (A)					2.94	0.124
Observation period (O)			0.30	0.583	3.70	0.055
S x O			9.42	**0.002**	12.60	**<0.001**
A x O					3.01	0.084

Significant effects are highlighted in bold

Apparent forage intake was neither affected by plot type, nor by animal species. Only a significant sward type*observation period-interaction ($P<0.05$) was found, showing a significantly higher amount of biomass taken up in grass-dominated swards in the winter observation period (71.8 g m^{-2}) than in the summer observation period (15.7 g m^{-2}). The biomass taken up in diverse swards was 78.1 g m^{-2} in summer and 69.5 g m^{-2} in winter observation period respectively (data not shown).

Forage residue (Fig. 1b) was significantly affected by the plot type. We found more biomass remaining at dung than at control plots. The forage residue was not affected by animal species. Like apparent forage intake, the forage residue was significantly affected by the sward type*observation period-interaction. More plant biomass was left in the grass-dominated swards in summer (196.1 g m^{-2}) observation period than in winter observation period (130.9 g m^{-2}). In the diverse swards, 149.9 g m^{-2} were left in summer and 163.8 g m^{-2} in winter observation period.

Vegetation height

Animal response is shown as the stubble height difference to the corresponding control plots. Only models including a significant effect of distance to the excrement on stubble height were included for further consideration (Table 3).

Table 3 Vegetation height of dung and urine plots adjusted for the height of control plots as affected by distance along a transect from the excrement, sward type (grass-dominated or diverse) and observation period (summer or winter); stubble height: vegetation height at the end of the stocking period following the one during which excrement were deposited; values represent the results of the linear mixed effects models (F and *P*); main effects and interactions for which no values are presented were not included in the simplified final linear mixed effects model.

Effect	Stubble height							
	Cattle				Sheep			
	Urine		Dung		Urine		Dung	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Distance (D)	0.20	0.648	46.16	**<0.001**	3.97	0.050	11.00	**0.001**
Sward (S)	4.87	**0.029**	7.81	**0.006**	7.30	**0.0**		0.687
Observation period (O)	11.28	**0.001**	10.31	**0.001**	11.18	**0.0**		0.674
D x S	5.54	**0.020**						
D x O			9.94	**0.002**			4.83	**0.031**
S x O					27.15	**<0.001**	9.72	**0.002**

Significant effects are highlighted in bold

Stubble height at cattle urine and dung plots was affected by distance from the excrement patch (Fig. 2a, b). The urine plots showed a significant distance*sward type–interaction, as less vegetation was left in the centre of grass-dominated swards for both observation periods. The diverse swards were grazed to a similar height across the whole measurement transect. The distance effect at the dung plots of cattle differed between the two observation periods. In the summer observation period, cattle left a higher stubble, particularly in close vicinity to the patch. For the winter observation period the reaction was less pronounced, but still visible.

For the stubble height at the dung plots under sheep grazing, the distance*observation period–interaction was significant. While the avoidance of biomass in dung plots was clearly visible for the summer observation period, particularly in close vicinity of the dung patch, there was no such response in the winter observation period (Fig. 2c). No distance-effect and therefore no spatial grazing response was found at the sheep urine plots.

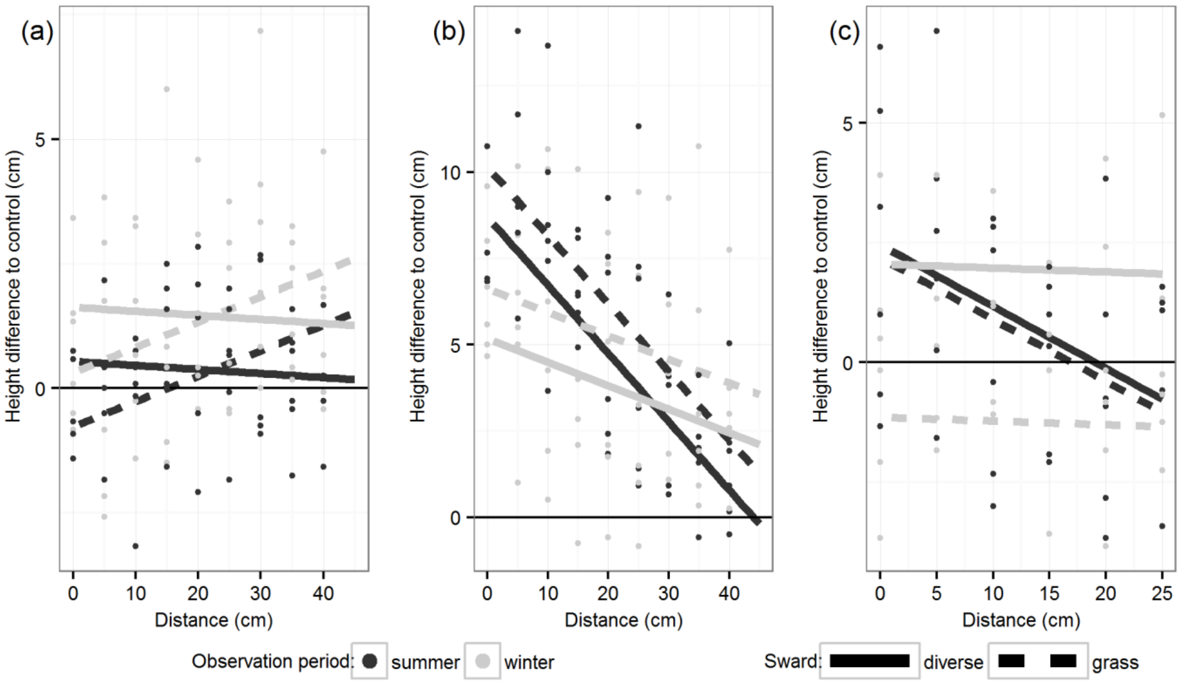

Figure 2 Vegetation height difference in relation to the control (no excrement) with distance from the excrement patches, vegetation stubble height following the stocking period on cattle urine plots (a), cattle dung plots (b), sheep dung plots (c).

Nutrient concentration and nutrient uptake of plant biomass and apparent animal nutrient intake

The statistical analysis of the nutrient concentration in sampled plant biomass of the first cut is shown in Table 4 and Figure 3a. The mean N and K concentration in plant biomass was 17 g kg^{-1} and 23 g kg^{-1}, respectively.

The N uptake of plant biomass was significantly increased (Fig. 3b) at urine compared to control plots. Furthermore, the K uptake (Fig. 3c) of sampled biomass was significantly increased, compared to the control, by both dung and urine. The P uptake was not affected by the different excrement plots.

The apparent N intake by grazing animals (Fig. 3d) was significantly higher at the urine plots in comparison to the control and dung plots. Furthermore the apparent K intake (Fig. 3e) was significantly higher at the urine plots. The different plot types had no effect on the P intake.

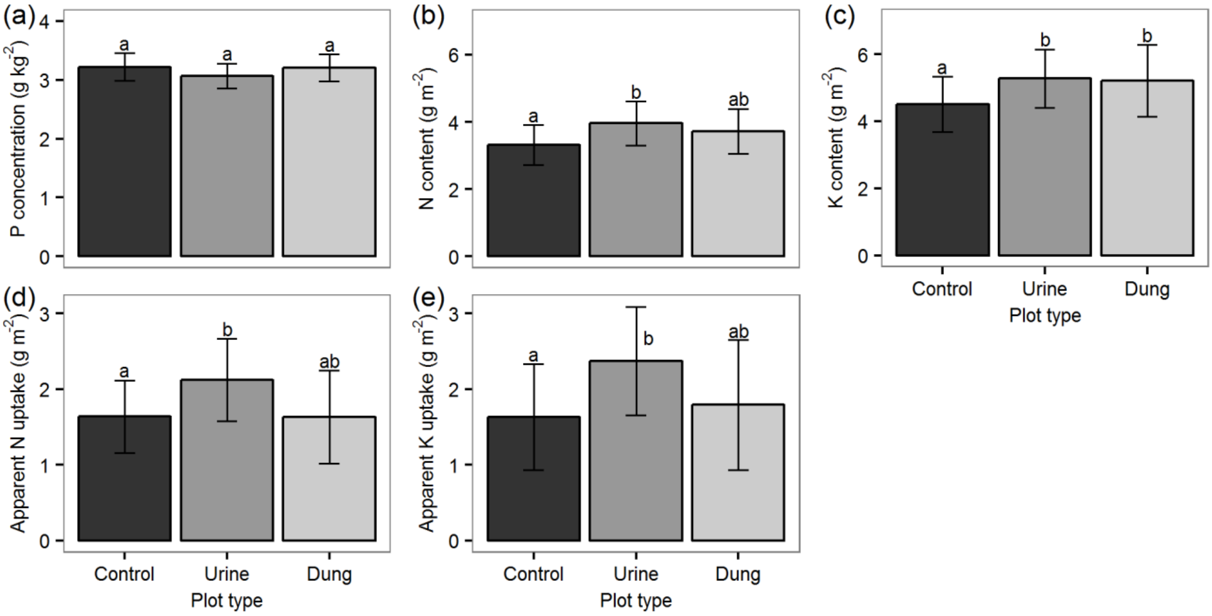

Figure 3 Mean (± standard error, *n* = 72) plant biomass P concentration (a) N uptake (b), K uptake (c), apparent animal N intake (d) and apparent animal K intake on different plot types, averaged across grazing animal species, observation periods and sward types. Means with different letters indicate significant differences between patch types (linear mixed effects models with Tukey LSD test at $P<0.05$).

Table 4 Effects of plot type (urine patch, dung patch, control) on the plant nutrient concentration, plant nutrient uptake and apparent animal nutrient intake, as affected by grazing animal species (cattle or sheep), sward type (grass-dominated or diverse) and observation period (summer or winter); values represent the results of the linear mixed effects models (F and *P*); main effects and interactions for which no values are presented were not included in the simplified final linear mixed effects model.

Effect	Nutrient concentration						Nutrient content in plant biomass						Apparent animal nutrient uptake					
	N		P		K		N		P		K		N		P		K	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Plot type (P)			3.36	**0.036**			3.65	**0.027**	2.50	0.084	4.10	**0.018**	3.22	**0.042**			3.85	**0.023**
Sward (S)	1.41	0.278	23.20	**0.001**	8.92	**0.017**	2.77	0.134					4.66	0.062	5.11	0.053		
Animal (A)	0.10	0.753																
Observation period (O)	104.92	**<0.001**	73.81	**<0.001**	3.70	0.055	21.85	**<0.001**	4.72	**0.031**			5.83	**0.016**	3.74	0.054		
P x O													2.85	0.060				
S x A	0.01	0.896																
S x O	7.59	**0.006**	13.75	**<0.001**	11.63	**<0.001**									8.02	**0.005**		
A x O	11.54	**<0.001**																
S x A x O	5.40	**0.021**																

Significant effects are highlighted in bold

Discussion

Effect of excrement on plant biomass production, nutrient concentration and nutrient uptake

Contrary to our first hypothesis, there was no interactive effect between the patch types and the other experimental factors on either biomass production, nutrient concentration or nutrient uptake. Urine deposition led to an increased plant biomass production as well as increased biomass N and K uptake. However, plant biomass production at these patches, which usually represent an input of highly plant available nutrients and thus distinctly increase plant biomass (Selbie *et al.*, 2015), was much lower than found in other studies. Average increases of plant biomass production by 25-40 % relative to the control have been reported for urine patches of cattle and sheep (Marsden *et al.*, 2016; White-Leech *et al.*, 2013a; Williams & Haynes, 1994). Similarly, the N concentrations of 17 g kg^{-1} found in the herbage growing at the excrement patches were far below the values of 21-43 g kg^{-1} reported by others (Moir *et al.*, 2013; White-Leech *et al.*, 2013b), whereas the K concentration of 23 g kg^{-1} was within the range reported (Kayser *et al.*, 2007). Dung patches neither affected biomass production nor N uptake, even though the decomposition of the dung patches during the winter observation period implied a possible mineralization of organic N in dung and hence an increase of plant available N (Deenen & Middelkoop, 1992; Haynes & Williams, 1993). The increase in plant K uptake and a slightly higher plant biomass P concentration in the dung plots are in line with other findings and can be ascribed to a high quantity of plant available inorganic P and soluble K in dung (Aarons *et al.*, 2009; Whitehead, 2000).

Assuming average nutrient concentrations of 5 g l^{-1} N and 8 g l^{-1} K in urine, 2 l urine per cattle and 0.2 l urine per sheep urine patch (Haynes & Williams, 1993), cattle and sheep urine plots would have received 10 and 4 g m^{-2} N and 16 and 6.4 g m^{-2} K, respectively. Similarly, assumed dung nutrient concentrations of 29 g kg^{-1} N, 12 g kg^{-1} P and 8 g kg^{-1} K (Haynes & Williams, 1993) and average dung patch weights of 2000 g for cattle and 100 g for sheep (Haynes & Williams, 1993) imply nutrient inputs of 58 g m^{-2} N, 24 g m^{-2} P and 16 g m^{-2} K on cattle and 2.9 g m^{-2} N, 1.2 g m^{-2} P and 0.8 g m^{-2} K on sheep dung plots, respectively. The weak plant productivity and nutrient uptake responses to urine and dung suggests that these nutrients were not efficiently used by the plants. Potential reasons include species composition, growth limitation caused by nutrients other than N, P or K and weather conditions that either limited plant growth or promoted nutrient losses.

Moir *et al.*, (2013) found great differences between thirteen common grass species in their ability to utilize the high level of N input found in urine patches. For the three most frequent species in our study (see Online Resource 2), they observed an above average shoot biomass increase in response to N application for *Dactylis glomerata*, while N response of *Poa pratensis* was below average, and that of *Lolium perenne* depended on the cultivar used (Moir *et al.*, 2013). Even though all three species are high-yielding grasses that are frequently sown in leys (Kirwan *et al.*, 2007), the genotypes at the study site do not represent commercially used cultivars. They may be adapted to relatively low levels of nutrient availability and less able to exploit high nutrient inputs.

At the experimental site, other nutrients than N, P or K are unlikely to limit plant growth. Boron (0.01 mg g^{-1}) and sulphur concentration (1.34 mg g^{-1}) measured in plants on an adjacent plot with the same management histories (unpublished data) as well as soil pH and soil extractable Mg concentrations point towards a sufficient availability of these elements.

Weather was generally favourable for plant growth during the two observation periods. Monthly average temperatures were very close to long-term averages from May to July in both years and 1-2 °C warmer than the long-term average in the months January to April 2014 and September 2014 to January 2015. Together with average or above long-term average precipitation from January to July 2014, this provided good growing conditions during the summer observation period. Only for the winter observation period, sub-average precipitation in the two months preceding the sampling might have limited biomass production. High precipitation rates directly following the excrement deposition in spring and autumn could have increased N leaching below the active root zone (Cameron *et al.*, 2013) and thus lowered the plant available N at the excrement patches. Additionally, nutrient leaching losses over winter usually lead to a seasonal variation in pasture reaction to the excrement in the following year (Moir *et al.*, 2011). Precipitation from October 2014 to March 2015 was 439 mm, which was 38 % higher than the long-term average, potentially increasing N leaching over winter. Nevertheless, the effect of urine on nutrient uptake did not differ between summer and winter observation periods. A closer consideration of nutrient losses at the excrement patches may be an approach for further investigations on the fate of nutrients.

Leaching may partly explain the lack of differences in urine effects between cattle and sheep. Given the sampling plot size chosen in our study, the per-area amount of nutrients returned to the pasture should have been higher at cattle than at sheep urine plots (see above) and thus should have resulted in a higher plant biomass production. A greater potential of the larger cattle urine patches to loose N (Orwin *et al.*, 2009) may have decreased animal-specific differences in plant biomass N uptake at the urine plots.

Contrary to our first hypothesis, the response of biomass production, N concentration or N uptake to excrement deposition did not differ between diverse and grass-dominated swards. For low-N sites, legume dominance has been shown to decline due to N return in excrement (Ledgard, 2001), partly counteracting the effect of this N return on biomass production and N concentration. The legume content was low in the diverse sward of our study, but their higher herbage proportion should also have affected the N uptake at the excrement plots.

Biomass production, nutrient concentration and nutrient uptake were also characterised by a great variability within experimental treatments.

Most of the existing research on urine and dung effects has been performed under simulated pasture conditions using artificially placed excrement and, in some cases, artificial urine.

In contrast to these experiments, studying excrement effects in grazed pastures induces several additional sources of variation: Nutrient concentrations in cattle and sheep urine can range widely between 2-11 g l^{-1} N and 7-9 g l^{-1} K (Haynes & Williams, 1993; Whitehead, 2000), with considerable variation even within the same animal (Hoogendoorn *et al.*, 2010). Somewhat smaller ranges of nutrient concentration have been reported for dung of cattle and sheep, with 2.3-2.9 % N and 0.7-0.8 % K and 0.7-1.2 % P in dung DM (Whitehead, 2000). Additionally, both the amount of urine and dung per excrement deposition and the area covered by each excrement patch are highly variable for both cattle and sheep (Haynes & Williams, 1993). Finally, grazing selectivity leads to heterogeneous swards containing plants in different states of regrowth, which can be expected to vary in their reaction to nutrient input. The variability observed in our results gives a new insight into the complexity of pasture response to excrement under real grazing conditions.

The effect of excrement on animal grazing behaviour

In general, herbivores avoid grazing swards contaminated with faeces as a method of parasite avoidance (Smith *et al.*, 2009). Even though the forage residue (Fig. 1b) did not differ between animal species, vegetation height measurement in close vicinity to the dung patches revealed a distinct difference in dung avoidance between cattle and sheep (Fig. 2b, c). These findings partly confirm our second hypothesis on animals rejecting biomass around the dung patches. Grazing avoidance by sheep in close vicinity to the dung patches was particularly distinct during the summer observation period, whereas plots containing dung patches were grazed to a similar height at the winter observation period. Hutchings *et al.* (1998) found increasing biting depth of sheep near dung with increasing age of the dung patch and initial avoidance was referred to odour of the dung. As opposed to this the vegetation stubble height left by grazing cattle only slightly changed for the longer winter observation period in comparison to the shorter summer observation period and was thus very high in the close vicinity to the dung patch (Fig. 2b). The difference in grazing selectivity between the two animal species is in line with findings by Cuchillo Hilario *et al.* (2017).

Avoided dung patches represent a substantial input of nutrients, which can increase soil fertility over a longer period (Aarons *et al.*, 2009; Ma *et al.*, 2013). As a consequence to the grazing avoidance and soil nutrient enrichment at the dung patches, Gillet *et al.* (2010) found seasonal changes in vegetation structure and composition around dung patches in oligotrophic and mesotrophic mountain pastures. Yoshitake *et al.* (2014) hypothesized the spatiotemporal restriction of dung effects to contribute to the heterogeneous pasture structure. Cattle can therefore create more patchy swards than sheep (Nolan *et al.*, 2001), especially in the year following late grazing rotations. Moreover, the size of the rejected area around the dung patches may as well be affected by the animal stocking rate and hence the grazing intensity.

Unlike the plant biomass of the forage residue, the vegetation height measurement showed that cattle preferentially grazed at urine plots of grass-dominated swards in both observation periods. Apparent animal N and K intake in the following stocking period was also significantly higher at the urine plots than at the control and dung plots. The preference of recent urine patches by grazing animals was found in previous studies and was attributed to a higher quality and quantity of nutrients at urine compared to unaffected patches (Jaramillo & Detling, 1992). Altogether, our results indicated that cattle and sheep grazing a low-input pasture did not differ in their biomass intake at the dung plots on the plot scale, however animal differences in grazing selectivity were observable in close vicinity to the dung patches.

Conclusion

The results of this study indicated that although the effects of excrement patches on plant parameters were detectable, the magnitude of effects was small in this low-input pasture and was hardly modified by animal species and sward composition differences. Even though excrement patches represented the main nutrient source at our experimental site, the effect on plant biomass production and nutrient concentration was smaller than in previous studies. The urine deposition of both animal species led to increases in plant biomass production and N and K uptake during both summer and winter observation periods. That an effect on plant biomass parameters could still be observed seven months after urine deposition may emphasize the importance of urine patches in low-input pastures. The mass of the forage residue in the presence of dung was increased for both cattle and sheep during both observation periods, but the small-scale vegetation height measurement showed a difference in avoidance of dung patches by the grazers. We therefore conclude that the variable vegetation response to nutrients influences the effect of excrement patches in this system.

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Chapter II:

Plant biomass production and soil nutrient availability following different excrement application times in a low-input pasture

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Abstract

Productivity of low-input pastures strongly depends on nutrient recycling via excrements of grazing animals. In our study excrements of cattle and sheep, collected from an adjacent rotational grazing system, were applied. Simulated urine and dung patches of cattle and sheep were created in spring on either grass-dominated or diverse permanent grassland swards. Additionally, cattle excrements were applied on diverse swards in summer and autumn. These treatments were compared to an untreated control and a fertilized control, representing the mean nutrient return by grazing animals. Aboveground plant biomass was sampled twice following excrement application in spring and summer and once following autumn. Belowground biomass and soil nutrients were sampled following the last aboveground biomass sampling. Soil was sampled separately below and around excrements. Aboveground biomass was only affected following spring application. The untreated control had a higher biomass on diverse than on grass-dominated swards. Dung patches decreased biomass at diverse swards. Cattle urine patches increased biomass at grass-dominated swards. Belowground biomass was not affected for any application time. The soil nutrient status indicated the greatest differences between treatments following spring application. Here the soil nutrient concentration was higher for cattle than for sheep excrements. Cattle urine patches significantly increased soil nitrate, ammonium and potassium concentration, cattle dung patches additionally increased phosphorus concentration. Sheep dung and urine only affected soil ammonium and nitrate concentration. Excrement application time and sward botanical composition affected the vegetation response. High soil nutrient concentration following spring application showed that nutrients were not fully used by the vegetation.

Keywords: Soil nutrients; Pasture; Heterogeneity; Excrements; Low-input

Introduction

In extensively grazed semi-natural grassland systems nutrient cycling is primarily mediated by excrements of grazing animals (Rotz *et al*., 2005). Compared to high-input systems, these low-input systems are mostly nitrogen (N)-limited. Excrements induce high nutrient loadings to a relatively small area and therefore are considered as ‘hot spots’ of intensive nutrient cycling (Haynes & Williams, 1993). They are the main nutrient source for the pasture vegetation in these systems.

The nutrient partitioning between urine and dung causes different nutrient availabilities in urine and dung patches (Whitehead, 2000). Soil nutrient status changes rapidly within days following the deposition of urine (Shand *et al*., 2000) and over a longer period below dung patches (Aarons *et al*., 2009). The large input of immediately available N and potassium (K) with urine patches is far in excess of current plant requirements and can damage the vegetation root system below the patch (Shand *et al*., 2002). Parts of the urinary N may be lost due to volatilization as ammonia, but a higher proportion may be nitrified resulting in an accumulation of nitrate (NO_3^-), which can be plant available, if not lost by leaching. The degradation of dung patches can increase soil phosphorus (P) and K content under the patch, as most of the K in dung is present as water soluble cations (Haynes & Williams, 1993) and a high proportion of P is present in inorganic form (Whitehead, 2000; Aarons *et al*., 2009).

Plant biomass response to excrements can be influenced by a number of factors. Apart from possible nutrient losses following the excrement deposition (Cai & Akiyama, 2016), grazing animal size (Bakker *et al*., 2004), pasture botanical composition and the time of excrement deposition (Bélanger *et al*., 2015; Moir *et al*., 2016) may be important factors.

Cattle and sheep represent two grazer species differing in size and nutrient return with their excrements (Haynes & Williams, 1993). The size of excrement patches returned to the pasture can determine the nutrient availability at the patch scale (Orwin *et al*., 2009). More importantly, the animal diet alters nutrient concentration in the excrements and their partitioning between urine and dung (Selbie *et al*., 2015). The season of excrement application also alters the pasture production (Moir *et al*., 2011; Bélanger *et al*., 2015; Moir *et al*., 2016). On species-poor, grass-dominated pastures, the high N input from excrements has been shown to strongly affect plant biomass production (White-Leech *et al*., 2013a, b). On grass-legume pastures, excrement patches can decrease the proportion of legumes and the amount of biologically fixed nitrogen, which offsets the effect of excrement-N input (Vinther, 1998).

So far, most studies on pasture response to simulated excrement patches were performed on intensively managed and fertilized pastures (Williams & Haynes, 1994; Saarijärvi & Virkajärvi, 2009; White-Leech *et al.*, 2013a; Moir *et al.*, 2016). Compared to low-input pastures, the contribution of excrement patches to the total pasture nutrient supply is less important in these systems. To get a better understanding of the importance of animal excrements for the plant biomass of diverse low-input pastures, the experimental design should, as far as possible, provide conditions close to a real grazing system, regarding excrement patch size, application rate and differing times of application. Due to seasonal dynamics of grassland biomass growth and different dynamics of nutrient release from urine and dung, observation of excrement effects should cover a whole grazing season.

Against this background, a field experiment was performed on a low-input semi-natural grassland using natural excrements of animals grazing at the same experimental site. This paper aimed to identify the effects of excrements on the pasture soil and vegetation under realistic conditions. Cattle and sheep excrements were applied on grassland swards of different botanical composition (diverse and grass-dominated) in spring, as the grass-dominated swards were expected to show a higher response to a high nutrient supply. Cattle excrements were applied in summer and autumn on diverse swards. Aboveground biomass was sampled in the subsequent growing season. Belowground biomass and soil nutrient status were assessed at the end of the experiment, to determine the medium-term pasture response to the excrements following a complete grazing season. This will give further information on excrement-mediated nutrient cycling in low-input pastures.

Material and Methods

Overview

Timing of excrement application and biomass sampling in the experiment closely followed that of a grazing experiment established on immediately adjacent paddocks. This reference experiment was run with three consecutive stocking periods per year (spring, summer and autumn).

Experimental site

The experimental site is located in the Solling Uplands, Germany (51°46'47 N, 9°42'11 E). Mean temperature and precipitation during the experiment are shown in Fig. 1. The soil type is a Vertic Cambisol (World Reference Base of Soils), the texture is clayey/silty loam. The initial botanical composition was a long-term permanent grassland corresponding to a moderately species rich *Lolio-Cynosuretum*. The experimental area was part of a prior mixed grazing experiment of cattle and sheep which ran from 2007-2011 (Seither *et al*., 2014). Thereafter the site was neither stocked nor fertilizer was applied and forage was cut twice per year (July and September) to a height of 7 cm and removed.

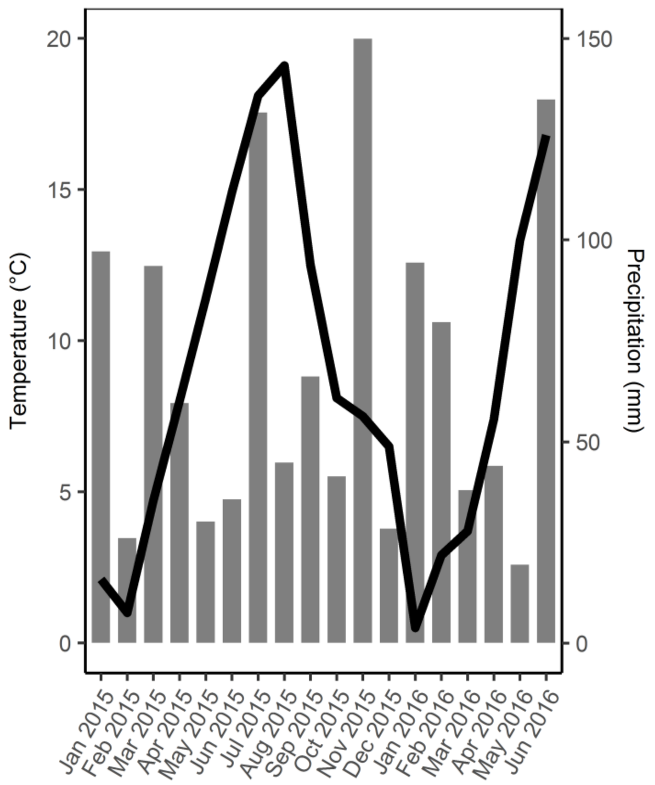

Figure 1 Monthly accumulated precipitation (mm) and mean temperature (°C) for the experimental years 2015 and 2016. Bars indicate precipitation and line indicates temperature.

Experimental design and treatments

The study area consisted of 64 square plots with a size of 1.5*1.5 m², split into 4 blocks. In autumn of 2014 6 plots per block were randomly chosen to manipulate the initial sward diversity by using herbicide against dicotyledonous plants (active components Fluroxypyr / Triclopyr), resulting in a low-diversity ‘grass-dominated sward‘ and an untreated ‘diverse sward’. In early spring 2015, the average contributions of plant functional groups to total biomass were visually estimated as 91% grasses, 8% herbs and 1% legumes at the grass-dominated swards, and 63% grasses, 36% herbs and 1% legumes at the diverse swards.

Fresh urine and dung were collected directly before each of the three applications (Table 1) from cattle (adult, non-lactating suckler cows of the breed German Simmental) and sheep (adult, non-lactating Blackheaded sheep ewes) rotationally grazing on either grass-dominated or diverse pastures at the reference experiment. On the excrement collection days, 5 cattle and 30 sheep from each sward-type were brought into the barn in the morning to collect the excrements during voluntary urination and defecation. The urine was immediately stored in sealed, cooled containers until its application. Excrements were kept separate for animals grazing on grass-dominated or diverse swards and were later applied at the corresponding sward-types. The excrement nutrient concentrations were determined as shown in Table 2.

Excrement application rates and sizes were based on Haynes & Williams (1993) and were 2 l for cattle or 0.15 l for sheep urine per plot. The urine was spread on the ground inside a metal ring with a diameter of 0.70 m for cattle and 0.42 m for sheep, which was placed in the centre of the plot to simulate the average surface area of a single urination. The dung plots received 2 kg of cattle or 0.1 kg of sheep dung (fresh weight), placed in the centre of the sampling area with a diameter of 0.3 m for cattle and 0.14 m for sheep. An ‘untreated control‘ and a ‘fertilized control’ were established. The fertilized control received 5 g N m^{-2} (calcium ammonium nitrate), 0.75 g P m^{-2} (triple superphosphate) and 5.7 g K m^{-2} (potassium chloride) at each of the three application times. Application rates were calculated to represent mean nutrient return by grazing animals based on the mean nutrient return through excrements in the reference experiment during the previous year. The treatments cattle urine, cattle dung, sheep urine, sheep dung, unfertilized and fertilized control were realized in this study. All plots were sampled within a sampling area of 1*1 m^2 for cattle and control and 0.5*0.5 m^2 for sheep, respectively.

Table 1 Overview over the course of the experiment, the experimental treatments and sampling times of aboveground, belowground biomass and soil.

Application time	Treatment	Sward type	Time of aboveground biomass sampling	Time of belowground biomass sampling	Time of soil sampling
spring application (01-08/06/2015)	cattle urine or dung	diverse	13/07/2015(summer) and 28/09/2015 (autumn)	20-21/10/2015 (autumn)	29-30/09/2015 (autumn)
	sheep urine or dung	diverse			
	cattle urine or dung	grass-dominated			
	sheep urine or dung	grass-dominated			
	fertilized	grass-dominated			
summer application (16/07/2015)	cattle urine or dung	diverse	28/09/2015 (autumn) and 07/06/2016 (spring)	20-21/06/2016 (spring)	08/06/2016 (spring)
	fertilized	diverse			
autumn application (30/09/2015)	cattle urine or dung	diverse	07/06/2016 (spring)	20-21/06/2016 (spring)	08/06/2016 (spring)
	fertilized	diverse			
no application	untreated	diverse	13/07/2015(summer), 28/09/2015 (autumn) and 07/06/2016 (spring)	20-21/10/2015 (autumn) 20-21/06/2016 (spring)	29-30/09/2015 (autumn) 08/06/2016 (spring)
	untreated	grass-dominated			

Table 2 Nutrient concentrations of the cattle and sheep urine (fresh matter basis) and dung (dry matter basis) for the spring application on grass-dominated and diverse swards. Characteristics of the excrements for the summer and autumn application on diverse swards. ND not determined.

Animal	Application time	Sward type	Urine			Dung		
			N (g l^{-1})	P (g l^{-1})	K (g l^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
cattle	spring 2015 (01-08/06)	diverse	4.7	0.011	1.84	23.6	10.7	7.8
cattle		grass-dominated	ND	ND	ND	21.4	9.4	14.4
sheep		diverse	1.6	0.002	7.40	27.3	12.4	18.9
sheep		grass-dominated	4.1	0.008	8.23	23.5	8.4	22.9
cattle	summer 2015 (16/07)	diverse	1.2	0.003	14.33	21.1	8.8	9.7
cattle	autumn 2015 (30/09)	diverse	1.4	0.003	4.07	20.3	9.2	12.9

Sample collection and analyses

Aboveground plant biomass was sampled in the two subsequent grazing rotations following application in spring and summer and in one rotation following excrement application in autumn. One half of each sampling area was randomly chosen at the first sampling date (Table 1) and the plant biomass was cut to a vegetation height of 2 cm using an electric scissors. The other half was sampled at the second sampling date. For the control plots one quarter of the sampling area was randomly chosen and in the subsequent sampling dates the other quarters were sampled. Following each sampling, the plant biomass of the unsampled parts of the sampling area and of the areas that received treatments at subsequent application times were cut to a vegetation height of 7 cm and plant biomass was removed. Sampled plant biomass was dried to constant weight for 48 hours at 60 °C to determine the dry matter (DM). For sampling areas with spring or summer application, DM of both sampling dates was summed up for further calculations to represent the accumulated biomass (Table 1).

Ten soil cores (diameter 1.5 cm) were collected in each plot from 0-10 cm depth either below the dung and urine patches (‘centre’) or distributed over the rest of the sampling area (‘edge’) following the last plant biomass sampling. Dung was removed before soil sampling below dung patches. The soil samples were analysed for their ammonium (NH_4^+) and nitrate (NO_3^-) concentration following extraction with KCl. Phosphorus (P) and potassium (K) concentration were analysed after extraction with calcium acetate lactate, using a continuous flow analyser (Seal Analytical, Norderstedt).

Belowground biomass was sampled shortly after the soil sampling. Per plot, 10 soil cores with a diameter of 1.64 cm were taken to a depth of 20 cm. The number of soil cores taken below the excrement patches corresponded to the proportion of the sampling area covered by the excrement patch. Roots were washed and dried to constant weight for 24 h at 30 °C to determine belowground biomass DM.

Statistical analyses

The statistical analysis was done using the program R version 3.2.0 (R Core Team, 2015). Influence of treatment and sward on the response variables were examined using linear mixed effects (LME) models and the package nlme (Pinheiro *et al*., 2015). For spring application, LME models had the fixed effects treatment (cattle urine, cattle dung, sheep urine, sheep dung and untreated and fertilized control) and sward type (grass-dominated and diverse) as well as their interactions. For summer and autumn applications, treatment (cattle urine, cattle dung, untreated and fertilized control) was the only fixed effect. All models considered the replicate blocks as random effects. Homogeneity of variance and normal distribution were checked for all residuals and a boxcox function was used to determine an appropriate transformation using the MASS package (Venables & Ripley, 2002). The initial models were simplified by removing non-significant interactions or factors, if this led to a lower value of second-order Akaike Information Criterion (AICc) of the resulting model. Pair-wise comparison of means was performed in case of significant fixed effects using the LSD test implemented in the package lsmeans (Lenth & Hervé, 2015).

Results

Above- and belowground biomass

Aboveground biomass was significantly affected by excrements following application in spring (Table 3). Here it showed a significant treatment*sward interaction. On grass-dominated swards the plots receiving fertilizer or excrements tended to have a higher accumulated biomass than the untreated control. Accumulated aboveground biomass was 59% higher on cattle urine patches in comparison to the unfertilized control, while the values at the other excrement patches did not differ significantly from the untreated control. On diverse swards the accumulated aboveground biomass following the cattle urine application was 16% higher than at the untreated control plots, but this difference was not significant. The application of cattle and sheep dung led to a 39% and 33% lower accumulated aboveground biomass compared to the untreated control. Furthermore, the accumulated biomass of untreated control plots was significantly higher (33%) on diverse than on grass-dominated swards. No significant treatment effects on the belowground biomass were measured for any application time.

Soil nutrient concentration

Following excrement application in spring, the NH_4^+ concentration in the centre of cattle dung patches was significantly higher than in the other treatments (Table 4). The edge sampling area of all excrement patches had a significantly higher NH_4^+ concentration than the untreated control plots. Treatment application in summer only increased the NH_4^+ content of the fertilized control. Here it was significantly higher than for cattle excrements at the edge sampling area. Following the autumn application, the NH_4^+ concentration of the edge sampling area was significantly higher at cattle urine and fertilized control plots than at the cattle dung plots.

The NO_3^- concentration in the centre and the edge sampling area was only significantly affected by excrements following spring and summer application. Following spring application, the NO_3^- concentration in the centre sampling area was significantly higher at plots receiving animal excrements than at the untreated control plots. The NO_3^- concentration in the centre sampling area was significantly higher on grass-dominated swards than on diverse swards. For the summer application the NO_3^- in the centre sampling area was significantly higher for both control plots compared to the cattle dung plots. Additionally the NO_3^- concentration at the edge sampling area was significantly lower for both cattle excrements in comparison to the fertilized control plots.

Table 3 Mean values (± standard error) and results of statistical analysis (*P* values) for above- and belowground plant biomass. Plant biomass dry matter (DM) for spring and summer application represents two consecutive cuts (accumulated biomass), plant biomass DM for autumn application only one cut. Means with different letters indicate significant differences between treatments within application date (linear mixed effect models with Tukey LSD test at P<0.05).

Application time	Treatment	Aboveground biomass DM (g m^{-2})		Belowground biomass DM (g m^{-2})
		Diverse	Grass-dominated	
spring	sheep urine	399±19^{bcde}	390±61^{bcd}	155±50
	sheep dung	315±45^{ab}	339±28^{abc}	128±35
	cattle urine	550±41^{e}	506±84de	258±101
	cattle dung	287±53^{a}	349±19^{abc}	142±27
	untreated	473±35de	319±19^{ab}	161±30
	fertilized	411±47^{bcde}	441±74^{cde}	211±44
P value				
treatment		<0.001		n.s.
sward		n.s.		n.s.
treatment*sward		<0.001		n.s.
summer	cattle urine	565±93	-	113±18
	cattle dung	405±40	-	128±33
	untreated	513±52	-	157±48
	fertilized	543±53	-	207±39
P value				
treatment		n.s.		n.s.
autumn	cattle urine	332±36	-	216±18
	cattle dung	331±33	-	165±37
	untreated	266±43	-	157±48
	fertilized	332±47	-	207±39
P value				
treatment		n.s.		n.s.

In soil samples taken following spring application, the highest extractable P concentration in the centre of the sampling area was found at dung patches. Soil P concentration remained unaffected following the summer and autumn application. Following spring application the extractable K concentration in the centre sampling area was significantly highest for dung and urine patches of cattle. The K concentration in the centre of the sampling area was also significantly higher on sheep urine than sheep dung plots. Moreover, there was a difference between the sward types, with higher K concentration on grass-dominated than on diverse swards, but this was only found for the centre sampling area. Following summer application of excrements, the cattle urine patches had a significantly higher K concentration in the centre sampling area than the untreated control. The cattle excrement application in autumn had no effect on the extractable K concentration.

Table 4 Mean values (± standard error) and results of statistical analysis (*P* values) for soil nutrient concentrations (dry mater basis; P, K: CAL extraction) under (centre) and around (edge) the excrement patches. Control plots were randomly sampled over the whole plot area. Means with different letters indicate significant differences between patch types within either centre or edge (linear mixed effect models with Tukey LSD test at *P*<0.05).

Application time	Treatment	NH_4^+ (mg kg^{-1})		NO_3^- (mg kg^{-1})		P (mg kg^{-1})		K(mg kg^{-1})	
		Centre	Edge	Centre	Edge	Centre	Edge	Centre	Edge
spring	sheep urine	2.9±0.3^{a}	3.0±0.4^{c}	2.4±0.7^{bc}	2.0±0.3^{b}	69.9±8.3^{a}	72.1±6.7	271.5±34.1^{b}	250.5±13.0
	sheep dung	3.1±0.6^{a}	2.9±0.4^{bc}	1.9±0.4^{b}	2.0±0.2^{b}	59.6±8.0^{a}	56.1±5.5	215.5±17.3^{a}	234.3±22.6
	cattle urine	2.9±0.4^{a}	2.6±0.3^{bc}	5.1±2.9^{c}	3.8±1.9^{b}	64.0±7.5^{a}	68.3±4.6	399.8±72.7^{c}	272.6±26.7
	cattle dung	3.3±0.7^{b}	2.7±0.3^{bc}	5.5±4.0^{c}	2.9±1.4^{b}	100.8±9.5^{b}	72.4±5.5	371.9±36.2^{c}	267.6±22.3
	untreated	2.1±0.1^{a}	2.1±0.1^{a}	1.3±0.1^{a}	1.3±0.1^{a}	66.2±9.1^{a}	66.2±9.1	231.0±26.4^{ab}	231.0±26.4
	fertilized	2.3±0.3^{a}	2.3±0.3^{ab}	1.9±0.4^{b}	1.9±0.4^{b}	67.0±13.5^{a}	67.0±13.5	245.1±30.8^{ab}	245.1±30.8
P value									
treatment		0.0017	0.007	<0.001	0.0012	0.0016	n.s.	<0.001	n.s
sward		n.s.	n.s.	0.0329	n.s.	n.s.	n.s.	<0.001	n.s.
treatment*sward		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
summer	cattle urine	3.8±0.3	3.5±0.2^{a}	1.8±0.6^{ab}	1.5±0.3^{ab}	64.1±10.2	71.6±10.9	305.6±17.7^{b}	284.4±25.9
	cattle dung	3.6±0.4	3.4±0.1^{a}	1.4±0.2^{a}	1.2±0.3^{a}	70.7±12.0	69.4±6.6	249.0±24.8^{a}	255.3±10.8
	untreated	3.8±0.1	3.8±0.1^{ab}	2.2±0.3^{b}	2.2±0.3^{bc}	57.3±8.5	57.3±8.5	212.0±19.2^{a}	212.0±19.2
	fertilized	5.0±0.7	5.0±0.7^{b}	2.6±0.3^{b}	2.6±0.3^{c}	69.4±8.5	69.4±8.5	252.3±27.0^{ab}	252.3±27.5
P value									
treatment		n.s.	0.0326	0.0028	0.0366	n.s.	n.s.	0.003	n.s.
Autumn	cattle urine	3.8±0.4	4.2±0.6^{b}	2.5±0.7	2.0±0.4	64.6±11.0	69.8±11.9	296.7±31.1	241.9±18.5
	cattle dung	4.0±0.4	3.3±0.2^{a}	2.2±0.4	1.4±0.3	155.3±54.2	76.7±3.5	300.8±42.8	223.9±18.0
	untreated	3.8±0.1	3.8±0.1^{ab}	2.2±0.3	2.2±0.3	57.3±8.5	57.3±8.5	212.0±19.2	212.0±19.2
	fertilized	5.0±0.7	5.0±0.7^{b}	2.6±0.3	2.6±0.3	69.4±8.5	69.4±8.5	252.3±27.0	252.3±27.0
P value									
treatment		n.s.	0.0371	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Discussion

Nutrient concentration of excrements

Nutrient concentration of excrements applied in our study showed great heterogeneity for the single application times (Table 2). They were distinctly lower than those reported by others (Haynes & Williams, 1993; Williams & Haynes, 1995; Shand *et al*., 2002), which could be attributed to the low-input management of the reference experiment (Haynes & Williams, 1993). Especially the urine N and K concentrations were widely ranging for single animal species and excrement application times. Additionally, nutrient concentration of cattle excrements tended to be lower for summer and autumn than for spring application. The excrement nutrient concentration of grazing herbivores varies depending on the quality of ingested forage (Whitehead, 1995). Urine N was previously reported to vary between and within days and within animals of the same species (Hoogendoorn *et al*., 2010). Thus under realistic grazing conditions both grazing management system and animal specific differences resulted in heterogeneous excrement nutrient concentrations, which consequently altered the amount of nutrients applied in our study.

Above- and belowground biomass

In the system used, treatments represented the main nutrient source for pasture vegetation growth, which should have led to high biomass response to the nutrients applied. However, aboveground biomass was only significantly affected following spring application of excrements with the highest biomass found at the cattle urine plots, which lasted over four months following application. Although replications of each treatment received the same amount of nutrients, the high standard error of aboveground biomass indicated a great heterogeneity. It appeared that nutrient application led to a higher increase of aboveground biomass at grass-dominated than at diverse swards. But even at the grass-dominated swards the effect of cattle urine on aboveground biomass was distinctly lower than reported by others for intensively managed pastures (Saarijärvi & Virkajärvi, 2009; White-Leech *et al*., 2013a). This implied a small response of aboveground biomass to the nutrients in our study.

Dung patches of both cattle and sheep had markedly negative effects on aboveground biomass at diverse swards. This negative effect was stronger following spring than for the later applications. For dung patches an initial negative effect on pasture vegetation, which can be followed by an increased production of vegetation surrounding the patches, was reported (MacDiarmid & Watkin, 1971; Aarons *et al*., 2009). Smothering of incompletely decomposed cattle dung patches could have had a great impact on vegetation (MacDiarmid & Watkin, 1971) in spring. Other than reported (Williams & Haynes, 1995; Ma *et al*., 2007) our findings imply a negative effect of sheep dung patches over four months on diverse swards.

Our results showed a very heterogeneous nutrient concentration of excrements and that the naturally developed diverse pasture influences the fertilizing effect of excrements under real grazing conditions. They moreover implied that the rotational grazing system induces a shift of nutrient availability within the grazing season, which does not necessarily overlap with times of high plant nutrient demand. However, other factors like warm conditions on the day of application in summer (20.1 °C) may have increased ammonia volatilization losses (Bolan *et al*., 2004) and nutrient leaching losses following autumn application, due to low plant nutrient demand (Saarijärvi & Virkajärvi, 2009), could have lowered the excrement effect on aboveground biomass. Against this, Bélanger *et al*. (2015) reported that cattle excrement application later in the year can increase forage production in the following year. But like other studies reporting high effects of excrements on aboveground biomass, their experiment was performed on recently resown pastures. As opposed to this, the grassland sward in our study developed naturally and thus was very old, which could have altered the pasture response to the nutrients. This assumption may be supported by the difference in production of the untreated grass-dominated and diverse control plots. In contrast to the results of Seither *et al*. (2014), untreated control had a higher forage production at diverse than at grass-dominated swards, whereas the grass-dominated swards showed a higher response to the nutrient supply. Furthermore the spatially homogeneous nutrient return of the fertilized control led to a differing pasture production of the sward-types.

No response of belowground biomass to the excrement application was found at the end of the respective grazing periods. Belowground biomass responded heterogeneously to the nutrient input at the plots for all application times. Apart from a possible root damage following application of nutrient rich urine patches (Shand *et al*., 2000; Shand *et al*., 2002), the nutrient addition to the N limited experimental site (Seither *et al*., 2014) should have caused root proliferation (Hodge, 2004). The results may imply that excrements do not affect belowground biomass in the low-input system over a long period.

Soil nutrient concentration

The soil nutrient sampling at the experimental plots was intended to give further information on the spatial pattern of the soil nutrient status at the end of the simulated grazing seasons and thus on the nutrient availability for subsequent plant growth. Treatments differed most strongly following spring application, where soil nutrient status differed distinctly between animal species and excrement patches. The NO_3^- concentration in the centre sampling area and the NH_4^+ and NO_3^- concentrations in both sampling areas of dung patches were significantly higher than at the untreated control. Rapid hydrolysis of urea in urine and subsequent nitrification (Williams & Haynes, 1994) promoted the NO_3^- concentration. However, high proportions of organically bound N in dung (White-Leech *et al*., 2013a) led to a continuous contribution of N to the soil, consequently increasing soil NH_4^+ concentration (Castillo *et al*., 2010). The NO_3^- concentrations were higher at cattle than at sheep urine patches. Due to the higher application rates and larger area covered by cattle, their patches retain N for a longer period than the smaller sheep urine patches (Orwin *et al*., 2009), which affects the further flow depth and thus N conversion in the soil (Williams & Haynes, 1994). All excrement patch types showed significantly higher NH_4^+ concentration at the edge sampling area than the untreated control, suggesting a possible nutrient leaching to the area surrounding the excrement patches.

The high urine application rates of cattle additionally increased the extractable K found following spring and summer application. Urine K can be adsorbed by soil exchange sites (Williams *et al.*, 1990), as soil and plant are the main sinks for urine K (Kayser *et al*., 2007). Furthermore the NH_4^+, extractable P and K soil concentration were significantly higher in the centre of cattle than sheep dung patches, which may be partly attributed to the difference in decomposition (Aarons *et al*., 2004), but was also affected by the generally higher dung application rates of cattle (Haynes & Williams, 1993). Additionally, the high content of soluble K in dung (Williams & Haynes, 1995; Aarons *et al*., 2004) led to a significantly higher extractable K under cattle dung patches in comparison to both control plots. The low soil nutrient concentrations following summer application could be caused by several heavy rain showers within the first 10 days following the application (in total 47.6 mm). This could have increased nutrient losses from urine and dung (McDowell, 2006; Saarijärvi & Virkajärvi, 2009).

Even following a whole grazing period, cattle and sheep excrements differed significantly in their effect on soil nutrient status. However, soil nutrient concentration was mostly significantly higher at the excrements and fertilized control than at the untreated control, indicating that pasture vegetation did not fully use the additional plant-available nutrients. Consequently grazing herbivores can contribute to increasing soil spatial heterogeneity in diverse grasslands (Liu *et al*., 2016), which may, in the longer term, lead to more heterogeneous swards (Gillet *et al*., 2010).

Conclusion

The aim of this experiment was to examine the relevance of excrement nutrients under conditions close to a real low-input grazing system. It showed that excrement nutrient concentration, plant biomass production and soil nutrient status of the pasture had a great variability. Even though representing the main nutrient source, the application of excrements did not always concur with plant nutrient demand and thus did not affect plant biomass for all application times. It can be concluded that the mid-term effect of grazing animal excrements at the patch scale mainly depended on the application time. Even a simulated uniform grazing and nutrient return, as represented by the fertilized control, had no consistent effect on the pasture and soil nutrient status. When upscaling this small-scale heterogeneity to the paddock, cattle excrements could lead to a higher spatial heterogeneity in soil nutrient status and consequently in plant biomass production in low-input pastures than sheep excrements (Liu *et al*., 2016), due to their more coarse nutrient return. Examination of nutrient losses at the excrement patches and an exact acquisition of plant species at the pasture would give further information on the heterogeneous forage response and soil nutrient concentration.

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Chapter III:

Small-scale sward heterogeneity rather than variable nutrient application rates in excrements determined plant nutrient concentration in a low-input pasture

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Abstract

In grazed pastures dung and urine patches of grazers provide variable amounts of nutrients for pasture vegetation growth. For low-input conditions there is no information on vegetation nutrient response to varying nutrient application rates with excrements. In this study urine and dung of cattle and sheep, derived from an adjacent rotational grazing system, were artificially placed on low-input diverse permanent grassland swards in spring. Cattle excrements were also applied in summer and autumn. Excrement nitrogen and potassium concentration were analysed prior the application. Additionally, untreated and fertilized swards, representing the mean nutrient return by grazing animals, were realized. Aboveground plant biomass was sampled according to the rotational grazing system and its nitrogen and potassium concentration was determined. Nitrogen concentration in urine was very low for all application times. Urine and dung showed a great variability of nitrogen and potassium concentration, which was independent from grazer species and excrement sampling time. Nitrogen and potassium concentration of plant biomass in untreated swards varied widely. Increasing nitrogen application rates with excrements significantly decreased the plant biomass nitrogen concentration. Potassium application rates did not influence plant biomass nitrogen concentration and plant biomass potassium concentration was unaffected by either different potassium or nitrogen application rates. The great variability of excrement and plant biomass nutrient concentration suggests that heterogeneity of biomass nutrient concentrations in low-input pastures may not necessarily be induced by the spatial heterogeneous excrement deposition of the grazers.

Keywords: Excrement; Low-input; Nutrient; Pasture; Response

Introduction

Grazing animals separate and concentrate nutrients within the small area of urine and dung patches (Afzal & Adams, 1992; Auerswald *et al*., 2010). Grazing animal species (Haynes & Williams, 1993), animal-specific characteristics (Hoogendoorn *et al*., 2010) and the quality of ingested forage (Whitehead, 2000) influence the nutrient concentration and thus the amount of nutrients returned with excrements. In low-input pasture systems, quality of ingested forage varies with grazing selectivity (Villalba & Provenza, 2009; Cuchillo Hilario *et al*., 2017) due to the botanically diverse sward composition (Rook *et al*., 2004). The separation of nutrients in urine and dung and the seasonally non-uniform deposition of the excrements furthermore alter the nutrient availability (Haynes & Williams, 1993; White-Leech *et al*., 2013; Bélanger *et al*., 2015). Consequently, in low-input systems, in which nutrient cycling is primarily mediated by excrements, excrements represent a variable nutrient supply for pasture vegetation.

The diverse botanical composition in low-input pastures (Rook *et al*., 2004) species-dependent differences in nutrient acquisition ability (Moir *et al*., 2013) and different stages of plant maturity present at the sward may alter the plant biomass nutrient response to excrements. Plant nutrient response to excrements can therefore be expected to be highly variable in the systems. To our knowledge there is no study investigating the response of plant biomass nutrient concentration to different nutrient application rates with excrements in a low-input pasture system.

We therefore performed an experiment simulating rotational stocking and excrement return on a low-input pasture. Excrements were derived from cattle and sheep rotationally grazing a low-input pasture with the same sward botanical composition and excrement nitrogen (N) and potassium (K) concentration was analysed. Experimental course closely followed this rotational stocking system. Cattle and sheep urine and dung were applied in spring and cattle excrements were applied in summer and autumn. In addition, an untreated control and a fertilized control representing the mean nutrient return of grazing animals were established. Aboveground plant biomass was sampled in the grazing rotation subsequent to the excrement application and its N and K concentration was analysed. The underlying hypothesis was that due to the low-input system, increasing N and K application rates with both urine and dung, will increase plant biomass nutrient concentration.

Material and Methods

The experimental area is situated in the Solling Uplands, Germany (51°46'47 N, 9°42'11 E) and its vegetation belongs to the plant association of a moderately species-rich *Lolio-Cynosuretum*. It consisted of 48 square plots split into four blocks, with a sampling area of 1*1 m² for cattle and 0.5*0.5 m² for sheep and control plots.

The treatments sheep, cattle urine and dung, untreated and fertilized control were realized. Times of excrement application and plant biomass sampling were based on a reference grazing experiment on nearby paddocks with the same sward botanical composition, which was run as a rotational stocking system with three rotations (spring, summer, winter). Sheep excrements were applied in spring and cattle excrements were applied in all stocking rotations. Directly before each application, fresh urine and dung were collected from cattle (adult, non-lactating suckler cows of the breed German Simmental) or sheep (adult, non-lactating Blackheaded sheep ewes) grazing the reference experiment. Excrement application rates and areas covered were chosen according to Haynes & Williams (1993) so the nutrient application rates with excrements were as shown in Table 1. The urine was spread inside a metal ring with a diameter of 0.7 m and 0.42 m for cattle (2 l) and sheep (0.15 l) respectively. Dung application rate was 2 kg of cattle dung or 0.1 kg of sheep dung, which was placed in the centre of the sampling area. The fertilized control received 15.0 g nitrogen (N), 2.25 g phosphorus (P) and 17.1 g potassium (K) m^{-2} as mineral fertilizer, equally shared between the three application times to represent mean nutrient return by grazing animals.

Table 1 Nutrient application rates for cattle and sheep urine and dung at three application times.

Animal species	Application time	Urine		Dung	
		N (g m^{-2})	K (g m^{-2})	N (g m^{-2})	K (g m^{-2})
Cattle	Spring 2015 (01-08/06)	9.4	3.7	7.0	2.3
Sheep		1.0	3.7	2.2	1.5
Cattle	Summer 2015 (16/07)	2.4	28.7	5.4	2.5
Cattle	Autumn 2015 (30/09)	2.8	8.1	8.8	2.7

Aboveground plant biomass was cut to a vegetation height of 2 cm using electric scissors at the next stocking rotation of the reference experiment that followed the excrement application. Thus the time of biomass sampling represented the next utilization of the pasture. K concentration of the samples was determined after digestion with aqua regia using an Inductively Coupled Plasma 6300 DUO ICP OMS (Thermo Fisher Corporation, Waltham) and N was determined according to the Dumas combustion method using a Variomax CN analyser (Elementar Analysesysteme GmbH, Hanau).

Statistical analysis

The statistical analysis was performed using the program R version 3.2.0 (R Core Team, 2015). The data for all application times were analysed as a combined dataset. Influence of N and K application rates with the excrements on N or K concentration in plant biomass was tested using linear fixed effect models implemented in the package nlme (Pinheiro *et al.*, 2015). The models had the fixed effects N or K application rate.

Results and Discussion

The nutrient application rates with urine and dung showed great differences between animal species and single application times (Table 1). Dung N and K concentration ranged between 20.3-27.3 g kg^{-1} and 7.8-18.9 g kg^{-1} (both N and K concentration on dry matter basis). In urine, 1.15-4.67 g l^{-1} N and 1.84-14.33 g l^{-1} K were found. A comparison of these data with concentrations reported by others showed that dung N and K concentrations in our study were within the range reported, but urine N concentrations, which showed by far the greatest variance, were much lower than reported average values (Haynes & Williams, 1993; Whitehead, 2000; Dijkstra *et al.*, 2013).

The N concentration in urine is influenced by forage N concentration, as the proportion of N excreted in urine increases with N concentration of ingested forage (Whitehead, 1995). An analysis of plant biomass nutrient concentration of the reference experiment at the times of excrement collection indicated of as little as 13.1 mg g^{-1} N and 24.6 mg g^{-1} K, which could have caused the low urine N concentrations (Whitehead, 1995). The impact of the diverse pasture in the reference experiment on grazing selectivity (Cuchillo Hilario *et al.*, 2017) and different stages of plant maturity present in these pastures could have altered the digestibility of herbage ingested and thus increased variability of excrement nutrient concentration (Bruinenberg *et al.*, 2002). So even though animals were grazing the same pasture, the nutrient concentration in urine and dung and thus the amount of nutrients returned via excrements by the 5 cattle and 30 sheep was very variable in the low-input system of the current study.

The statistical analysis on the plant N and K concentration response to different nutrient application rates with excrements was performed independently from time of application, excrement type and animal species in order to observe the general effect of nutrient application on plant biomass nutrient concentration. We generally found a great variability of plant nutrient concentrations within the different N and K application rates (Fig.1). Only the N application rate significantly affected the plant nutrient concentration, with increasing N application rates resulting in a significant decrease (*P*=0.0243) of the plant N concentration.

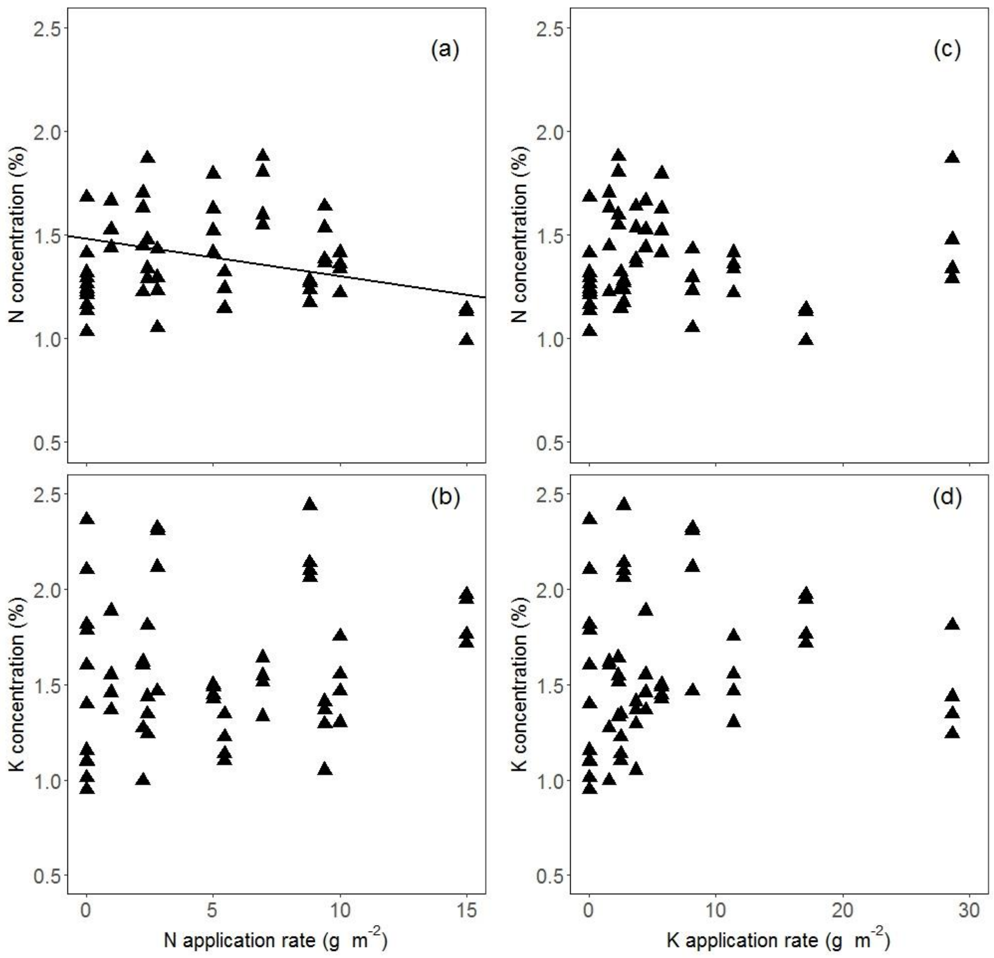

Figure 1. Effect of N application rate (g m^{-2}) on the plant biomass N (a) and plant K concentration (b). Effect of K application rate (g m^{-2}) on the plant N (c) and plant biomass K concentration (d). The line in figure (a) indicates the significant relationship ($P<0.05$) between N concentration in plant biomass and N application rate.

A high variability in plant nutrient concentration was measured even for the unfertilized control. Plant nutrient concentration therefore seemed to be independent from the general presence of excrements. As a result, the overall high variability of plant biomass N concentration may reduce the interpretability of the effect found for increasing N application rates. Thus the high variability in plant nutrient concentration found for the broad range of nutrient application rates falsifies our hypothesis.

In a grazed pasture, nutrient utilization from excrements and subsequent acquisition by plants is a complex process depending on both nutrient mineralization from excrements, influenced by form and time of excrement deposition (Whitehead, 2000; Bélanger *et al*., 2015), and on the current plant nutrient demand at the time of excrement deposition (Bélanger *et al*., 2015).

However, for a low-input pasture our results showed that these processes did not play a role at the small scale of the excrement patch. The results also suggest that the plant nutrient concentration was independent from the nutrient application rate. This leads to the hypothesis that the spatially heterogeneous sward structure in low-input pastures may not be entirely conditioned by soil spatial heterogeneity caused by grazing animals (Liu *et al*., 2016). The high variability of plant biomass nutrient concentrations found for the untreated control may support this hypothesis.

To our knowledge this is the first study observing plant biomass nutrient response to a broad range of excrement nutrient application rates in a diverse low-input pasture system. In conclusion, we found high variability for the excrement nutrient concentration and hence nutrient application rates, which may be even higher under real grazing conditions (Haynes & Williams, 1993). Plant biomass nutrient concentration varied strongly with little influence of nutrient application rates. Factors other than spatially heterogeneous excrement deposition seem to be important for small-scale variation of plant biomass nutrient concentration under low-input conditions.

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General Discussion

The aim of the present study was to evaluate the effect of grazing animal species and pasture sward botanical composition on nutrient cycling in a low-input semi-natural grazing system. For this purpose two field experiments were conducted: one grazing experiment and one simulating rather realistic grazing conditions (simulation experiment). These experiments were performed to enable an examination of excrement effects from different perspectives. Both the plant biomass production and the plant nutrient concentration on different sward types (diverse and grass-dominated) and grazing animal forage uptake (cattle and sheep) at urine and dung patches were analysed in a grazed pasture of the grazing experiment. Two observation periods were realized in this experiment: Grazing and excrement deposition in spring with regrazing in summer and grazing and excrement deposition in autumn with regrazing in spring of the following year. The course of the simulation experiment was adapted to that of the grazing experiment. Excrements from the grazing experiment were applied according to the stocking rotations of the grazing experiment. Cattle and sheep urine and dung were applied on both grass-dominated and diverse swards in spring, whereas cattle excrements were additionally applied in summer and autumn on diverse swards. Aboveground plant biomass production and nutrient concentration were sampled in the course of the grazing season. Additionally, soil nutrients directly below and around excrement patches and belowground biomass were sampled at the end of the season.

Nutrient cycling in grazed low-input pastures is mainly mediated by excrements of the grazing animals (Rotz *et al.*, 2005). The excrements alter the plant biomass production and nutrient concentration (Williams & Haynes, 1995; White-Leech *et al.*, 2013a; Marsden *et al.*, 2016) which results in areas being temporally favoured (Day & Detling, 1990; Steinauer & Collins, 2001) or avoided (Smith *et al.*, 2009; Gillet *et al.*, 2010). They are therefore the central point of nutrient cycling in this system.

I will now discuss the results of this study concerning the following questions: What effects do animal- and sward-specific responses to excrements have on nutrient cycling within the stocking period subsequent to different excrement deposition times? How strong is the medium-term effect of nutrient separation in urine and dung on plant biomass production and soil nutrient status? To what extent does the plant biomass nutrient concentration respond to varying excrement nutrient application rates present in a real grazing system?

In my investigation, the analysis of plant biomass data revealed that the excrement effect on plant biomass differed between the stocking period and a complete grazing season following the excrement deposition. In the grazing experiment (Chapter I) there was a general effect of urine patches significantly increasing the plant biomass production as well as N and K content and of dung patches significantly increasing the plant biomass K content. The results indicated no differences in the effect of excrements on plant biomass within the stocking period subsequent to the excrement deposition in spring and autumn. The stocking periods differed in terms of weather conditions and resting intervals between excrement deposition and plant biomass sampling. Other studies have shown clear differences in the plant biomass production following excrement application in spring and autumn (Moir *et al.*, 2011; Bélanger *et al.*, 2015), which could be attributed to a lower plant nutrient demand for excrement application times later in the year. Therefore, the missing difference in plant biomass response to the excrements between the two stocking periods led to the assumption, that plant nutrient uptake from excrements and thus plant productivity may be, to a certain point, limited by the ability of plant biomass to utilize the nutrients supplied in the low-input system of the current study. The fact that the effect of urine patches on plant parameters in this study was by far lower than it has been reported even for intensively used pastures (Williams & Haynes, 1994; White-Leech *et al.*, 2013a, b; Marsden *et al.*, 2016) may support this theory.

The plant biomass N and K concentration following the application of different N and K application rates through excrements (Chapter III) gave further explanation for the low response of plant biomass to the excrements. It showed a high variability of plant biomass nutrient concentration which was independent from the nutrient application rates through excrements. Even plant biomass N and K concentration of the untreated control varied greatly which implied that the very old, naturally developed grassland sward in this study may have caused the high variability at the small scale of the excrement patches. This may furthermore be an explanation for the missing effect of excrement deposition time on plant parameters sampled. An acquisition of the nutrient uptake ability of plant species present (Moir *et al.*, 2013) and shorter periods between excrement deposition and plant sampling (Moir *et al.*, 2011) would give further information on the low plant biomass response to excrements.

The analysis of the urine and dung nutrient concentrations (Chapter III) showed a generally high variability for both cattle and sheep excrements for different sampling times and sward types. Since the sampling area of cattle (1 m^2) and sheep (0.25 m^2) corresponded to the same ratio between the natural excrement patch and the rest of the sampling area, the missing difference in animal specific effects on plant production and nutrient concentration may be attributed to the lack of difference in excrement nutrient concentration (Hoogendoorn *et al.*, 2010). Moreover, it can be assumed that besides the nutrient concentration, the amount of excrements returned to the sward varied greatly as well throughout in the grazing experiment (Haynes & Williams, 1993; Whitehead, 2000). This could have promoted the variability found for the plant biomass nutrient concentration. The analysis of forage residue at the excrement patches, used as an indicator of grazer forage uptake at the excrements showed a significant increase in forage residue at the dung patches of both cattle and sheep following both excrement deposition in spring and autumn. In general grazers avoid grazing at dung patches (Smith *et al.*, 2009). The additional lack of preference for urine patches (Day & Detling, 1990; Steinauer & Collins, 2001) led to the assumption that within the stocking period, subsequent to the excrement deposition, plant biomass and thus nutrients at the excrement patches are not being reused by the grazing animals. Plant biomass is therefore rather left for decomposition and may thus be cycled through the litter cycle (Bardgett & Wardle, 2003). However, the difference in grazing avoidance in close vicinity to the dung patches, which was stronger for cattle than for sheep, as indicated by the vegetation height measurement, may lead to a change in composition and structure of vegetation growing at cattle dung patches (Gillet *et al.*, 2010). This depends on the period of dung rejection, which was reported to last up to 18 months for cattle (Haynes & Williams, 1993).

Considering the second question (Chapter II), the cattle urine patches significantly increased the plant production on grass-dominated swards following application in spring. These findings are in line with other studies reporting a positive effect of cattle urine patches on plant production up to four months following the application (White-Leech *et al.*, 2013b) and multiple cuttings (Bélanger *et al.*, 2015). The significantly increased soil NO_3^- concentration at cattle and sheep urine patches indicated that the urine N supply exceeded the limited plant biomass N uptake over the season. The dung patches of cattle and sheep had no negative effect on plant production on grass-dominated swards. Jørgensen & Jensen (1997) reported that in grass-legume mixtures the N, mineralized from dung, was predominantly taken up by grass, which may be an explanation for the increased plant production on grass-dominated swards in the current study. The application of dung patches of both cattle and sheep on diverse swards resulted in a negative effect on plant production. For cattle dung patches a negative effect on plant production over 112 days has been reported (White-Leech *et al.*, 2013b). Therefore the results of the current study indicated that dung patches did not only affect the area covered (Dai, 2000), but also the vegetation surrounding the dung patches. Sheep dung patches were completely decomposed and significantly increased soil NO_3^- concentration, which implied a mineralization of inorganic N in dung and hence an increase of plant-available N (Deenen & Middelkoop, 1992; Haynes & Williams, 1993). In contrast to other studies (Williams & Haynes, 1995; Ma *et al.*, 2007) the findings of the current study indicated a negative effect of sheep dung on plant production for a period of four months following application. The dung patches of cattle represented a source of extractable P and K directly below the patch and also for NH_4^+ and NO_3^- in both sampling areas at the end of the grazing season which indicated that a mineralization of N took place in the course of the experiment (Deenen & Middelkoop, 1992; Haynes & Williams, 1993). The adverse effect of dung smothering on grass growth may have lowered the plant nutrient uptake (Cai & Akiyama, 2016).

Cattle urine and dung application later in the year (summer and autumn) did not affect the production of subsequently growing plant biomass. This was similar to the application in spring where the effect of cattle excrements was also small on the diverse sward. Missing differences of soil N concentration between excrements and untreated control implied that N from excrements were lost, for example by leaching over winter (Saarijärvi & Virkajärvi, 2009), rather than utilized by pasture vegetation. Due to the experimental procedure of the simulation experiment, nutrient losses could not be quantified. However, decomposition of dung in the following winter implied N mineralization (Deenen & Middelkoop, 1992; Haynes & Williams, 1993), yet the soil N status was not affected. Thus the results indicated that in rotationally grazed low-input systems the excrement deposition by grazers and current plant nutrient demand did not necessarily overlap. Moreover, the differences in nutrient availability of dung and urine (Whitehead, 2000) did only affect plant production on grass-dominated swards.

My investigation has shown that the sward botanical composition, the grazing animal species and the time of grazing did not alter the effect of urine and dung patches on plant biomass within the stocking period subsequent to the excrement deposition. In terms of the excrement effect throughout a whole grazing season, grass-dominated patches caused a higher productivity at cattle urine patches. The results showed an overall low response of plant biomass production to excrements and moreover indicated a high variability in plant nutrient concentration which may be an explanation for the heterogeneous sward structure of the low-input system used in this study. Additionally, the higher grazing avoidance by cattle than by sheep in close vicinity to the dung patches increases this heterogeneity. Further investigations of nutrient losses at the excrement patches as well as shorter periods between excrement deposition and plant biomass sampling (Moir *et al.*, 2011) would give more information on nutrient cycling in this system.

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Summary

Excrements of grazing animals play a key role for nutrient cycling in grazed grasslands. So far, most studies on nutrient cycling in pasture systems were performed using simulated excrement patches under simulated grazing conditions. Influences of both plant and animal functional traits on nutrient cycling are known for soil-to-plant-to-animal cycling steps, but relatively little is known about their effect on animal-to-soil-to-plant cycling steps. In low-input pastures excrements from grazing animals represent the main nutrient source for grassland vegetation. Plant and animal functional traits should therefore have a greater impact on nutrient cycling in these systems. For this approach the excrement patch is the functional scale at which nutrient cycling should be assessed in a grazed grassland.

In this study, I investigated grazing animal excrement patches to determine whether different plant and animal functional traits have an effect on plant biomass production, plant nutrient concentration and grazing animal selectivity under real grazing conditions in a grazing system with rotational stocking. Furthermore, I studied the medium-term effects of grazing animal excrements on the plant biomass, plant nutrient concentration and soil nutrient concentration under simulated conditions over a complete grazing season.

The experiments were performed in a semi-natural grassland, which corresponded to a moderately species-rich *Lolio-Cynosuretum* community. The site is located in the Solling Uplands (Germany / Lower Saxony). Two sward types were realized: a moderately species-rich (diverse) and a grass-dominated sward, achieved by selective herbicide application. In a grazing experiment, swards were rotationally grazed by cattle or sheep from spring to autumn. Excrements were marked in spring and autumn and plant biomass and vegetation height were sampled in the subsequent grazing rotation to cover both plant and animal response. Plant biomass nutrient concentration, production and grazing animal forage uptake at the excrement patches were not affected by the stocking periods, animal species and sward types in the grazing experiment. There was only a general effect of excrement patches on these factors. The results indicated an overall low response of the grassland vegetation to the excrements. Presence of urine patches increased plant biomass production as well as the nitrogen and potassium content of the forage. Dung patches increased the plant biomass potassium content and decreased the animal forage uptake. Regarding their gracing behaviour, clear differences could be seen between cattle and sheep in close vicinity to the dung patches. Cattle avoided grazing vegetation

close to dung patches following both deposition in spring and in autumn and sheep only following dung deposition in spring. Furthermore, cattle preferentially grazed at urine plots of the grass-dominated swards.

A second experiment was realized with simulated grazing where excrements, derived from the grazing experiment, were applied and plant biomass was sampled according to three grazing rotations over the year. Additionally, soil nutrient status was sampled at the end of the simulated grazing season to cover the medium-term pasture response to excrements. In this simulation experiment, the excrements only affected the plant biomass production following application in spring without differences between animal species. Grass-dominated swards showed a higher production than diverse swards at plots receiving excrements. Dung patches decreased plant biomass production at the diverse swards and cattle urine patches increased plant biomass production at grass-dominated swards. Excrement nutrient concentration showed a great variability for both cattle and sheep and sampling date. The analysis of plant biomass nutrient concentration at the simulation experiment revealed a great variability of both plant biomass N and K concentration which was independent from the excrement nutrient concentration, time of excrement application and sward botanical composition. Soil nutrient status showed the greatest response to the application of excrements in spring. Cattle induced a higher soil nutrient concentration (nitrate, ammonium, potassium) than sheep. Cattle dung patches additionally increased the phosphorus concentration. Sheep dung and urine patches only affected the soil nitrate and ammonium concentration.

Our results indicate an overall low response of plant biomass parameters to the presence of grazing cattle and sheep excrements, whereby plant and animal functional traits did not alter the plant response at the excrement patch. In the low-input pasture, pasture vegetation and grazing animals showed a rather general response to the presence of excrement. The high variability found for excrement and plant biomass nutrient concentration and plant biomass production indicates the complexity of nutrient cycling in a real grazing system. At the small-scale of the excrement patch, this high variability is an important factor determining nutrient cycling. Assessing nutrient losses following excrement application and determining the nutrient use efficiency of pasture vegetation at the excrement patch scale should give more detailed information on nutrient cycling.

Presentations and Publications

Scheile, T., Isselstein, J., Tonn, B. 2015. Beeinflussung des Biomassewachstums sowie der Selektivität von Weidetieren durch Exkrementstellen bei extensiver Beweidung. Mitteilung der Arbeitsgemeinschaft Grünland und Futterbau 16, 144-177.
Including a poster presented at the AGGF 2015 in Aulendorf, Germany.

Scheile, T., Isselstein, J., Tonn, B. 2016. Einfluss von Exkrementstellen auf die Biomasseproduktivität sowie die Selektivität von Weidetieren bei extensiver Beweidung. Mitteilung der Arbeitsgemeinschaft Grünland und Futterbau 17, 97-99.
Including a poster presented at the AGGF 2016 in Luxembourg City, Luxembourg.

Scheile, T., Isselstein, J., Tonn, B. 2016. Effect of excreta patches on biomass productivity and grazing selectivity in low-input pastures, Grassland Science in Europe 21, 470-472.
Including a poster presented at the EFG 2017 in Trondheim, Norway.

Scheile, T., Isseltstein, J., Tonn, B. 2018. Herbage biomass and uptake under low-input grazing as affected by cattle and sheep excrement patches, *Nutrient Cycling in Agroecosystems,* doi:10.1007/s10705-018-9945-3.

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