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# Nitrogen excretion and utilisation of dairy cows grazing temperate semi-natural grasslands

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ABSTRACT

Diets reliant on grazed, temperate herbage are prone to greater nitrogen (**N**) losses via urine than balanced stall-fed diets which poses a greater risk for N emissions. Measures for improving the N utilisation in grazing-based dairy cattle systems are predominantly investigated on homogenous clover-ryegrass pastures with high herbage yields and nutritional quality. In contrast, grazing-based systems reliant on less external inputs (e.g., synthetic fertilisers or concentrates) using semi-natural grassland as main feed source, such as in large parts of Central Europe, received less attention. The N utilisation and excretion of grazing cows in low-input dairy farms were, thus, investigated on nine commercial organic dairy farms in South Germany across one to four periods per farm. The dataset captured a diverse set of dairy production systems comprising 323 individual animal observations. A mean (± one SD) milk production, DM intake (**DMI**), and pasture DMI of 23.9 kg (± 5.35), 21.0 kg (± 3.21), and 11.3 kg/d (± 4.83), respectively, was determined. Feed intake was estimated using titanium dioxide and faecal CP concentration as markers of faecal excretion and diet digestibility, respectively. Milk N use efficiency (**MNE**; i.e., milk N secretion as share of N intake) averaged 24.7 g/100 g N intake (± 5.91), which is greater than observations in temperate, high-input grazing systems but lower than in cows receiving balanced diets in the barn. The MNE and another seven indicators of N utilisation and excretion displayed a wide range of values. The grazing management factors explaining this variation were, thus, identified via backward elimination. The supplementation strategy had the greatest potential for manipulating N utilisation and excretion of dairy cows. Increasing shares of fresh forages (i.e., meadow grass or clover-grass leys) as well as of hay in supplement DMI increased N utilisation (e.g., MNE) and decreased urinary N excretion (e.g., urinary N to creatinine ratio), while increasing shares of concentrates in supplement DMI are related to lower N losses via urine. At the same time, increases in total supplement DMI reduced N utilisation and increased urinary N excretion. Hence, full-time grazing combined with supplementation of fresh forage and hay in the barn is a viable option for low-input, grazing-based dairy operations with moderate levels of N losses.

## Implications

Dairy cattle systems contribute substantially to environmentally harmful nitrogen emissions via animal excretions. These emissions are potentially greater and more variable under grazing conditions than in stall-based feeding systems due to the high concentration of nitrogen in fresh grasses. The present study investigated the magnitude of nitrogen excretions in low-input grazing systems and the related grazing management factors. Low-input grazing systems, such as organic dairy farms, utilised the feed nitrogen more efficiently than high-input grazing systems. The type of supplement feeds offered to grazing cows had the greatest potential for manipulating their nitrogen utilisation and excretion.

## Introduction

A major challenge in grazing-based dairy cattle feeding is the generally less efficient utilisation of dietary nitrogen (**N**) for milk production, leading to a greater risk for atmospheric and hydrospheric N emissions than with a balanced diet fed in the barn (Hoekstra et al., 2007; Huhtanen et al., 2015). Nitrogen utilisation

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for microbial and milk protein synthesis is predominately determined by N intake, rumen degradability of ingested CP, and dietary CP-to-energy ratio (e.g., Hoekstra et al., 2007; Li et al., 2013; Schuba et al., 2017). Grazed, temperate herbage contains high concentrations of readily degradable CP, but commonly has low concentrations of fermentable carbohydrates, which increases ammonium absorption from the rumen and thus urinary N excretion (Hoekstra et al., 2007). This high urinary N excretion increases the risk of nitrate leaching, in particular from urine spots on pastures, because available N exceeds plant nutrient requirements (Selbie et al., 2015). Faecal N, on the other hand, is present in a more stable form, decreasing the risk for environmental losses on pasture so that a greater faecal:urinary N ratio is desirable (Totty et al., 2013). The substantial spatial and seasonal heterogeneity in the availability and nutritional value of pasture herbage challenges the scope of compensating for the excessive rumen-degradable CP supply from pasture, for instance, by grazing management. It is, thus, necessary to identify grazing management factors, including paddock management factors (i.e., stocking density, herbage allowance and mass, timing of grazing, and nutritional quality and botanical composition of pasture herbage) and supplementation strategies, which effectively reduce urinary N excretion and increase N utilisation for microbial and milk protein synthesis.

The effects of such grazing management factors on N utilisation and urinary N excretion of grazing cows have been investigated mainly in high-input grazing systems using homogenous clover-ryegrass pastures with high biomass yields and nutritional quality of herbage (Hoekstra et al., 2007; Dodd et al., 2019). In contrast, grassland-dominated, low-input farming systems, including organic dairy farms, using semi-natural grassland as the main feed source, have received less attention in research so far (Akert et al., 2020). Semi-natural grasslands are multispecies, permanent grasslands with no to little treatment with fertilisers, pesticides, or soil cultivation, and thus, at least in part, with lower yields and nutritional quality of herbage (Bruinenberg et al., 2003; Peeters et al., 2014). This type of grasslands can constitute the main feed resource in regions with low mountain ranges or in (pre-)alpine regions where less favourable soil and topographic conditions signify a high share of permanent grasslands. Moreover, in low-input, grassland-dominated farming systems, supplementation of grazing cows frequently consists of freshly cut meadow grass from permanent grasslands or of freshly harvested grass-clover mixtures originating from the farm's cropland.

It was hypothesised that N utilisation in such dairy production systems is lower and urinary N excretion is greater than in high-input grazing or stall-fed systems, due to high herbage intakes and limited concentrate feeding, increasing the imbalance between ruminal N and fermentable carbohydrate supply (Akert et al., 2020; Correa-Luna et al., 2020). Further, the lower feed intake and performance level of dairy cows in such systems are typically related to a lower resource use efficiency – at least on the production level (e.g., conversion of ingested DM into milk) (Capper and Bauman, 2013). Yet, differences in N utilisation and excretion are also expected between different low-input grazing systems due to variation in grazing and supplementation intensity. For instance, milk N use efficiency (**MNE**) is likely lower in cows grazing full-time than in those that are grazing part-time and are supplemented with more mature fresh forages with lower CP concentrations than pasture herbage (Akert et al., 2020).

Therefore, the present study aimed at (1) to quantifying the N excretion and utilisation of lactating cows grazing temperate, semi-natural grasslands in low-input dairy systems using eight N-related indicators, and (2) exploring the effect of various grazing management factors on N utilisation and excretion of cows within these systems. Preliminary results from the present study were previously published as abstracts (Perdana-Decker et al., 2023b, 2023c).

## Material and methods

### *Study farms and setup*

The present study is based on a dataset collected on nine commercial, organically led dairy cattle farms located across five natural regions in Southwest Germany (i.e., Schwäbische Alb, Schwäbisches Keuper-Lias-Land, Donau-Iller-Lech-Platte, Voralpines Hügel- und Moorland, and Hochschwarzwald). Sampling was repeated in four sampling phases from May to October of 2019 and 2020. Phase 1 took place from May to July 2019, Phase 2 from August to October 2019, Phase 3 from May to July 2020, and Phase 4 from August to October 2020 (Table 1). Within each sampling phase, multiple farms were visited consecutively for examination periods of 11 d each. These examination periods consisted of 5 d of adaptation for feeding the external faecal marker titanium dioxide (**$TiO_2$**) followed by 6 d of sampling of faeces, milk, urine, standing pasture herbage on offer, and supplement feeds. Four farms were visited in every sampling phase, five farms only during the two phases of 2020, and one farm was only visited in Phase 4.

For animal-related data, 10–28 lactating dairy cows per farm and phase were selected for sampling depending on the herd size (Tables 1 and 2). In Phases 3 and 4, cows were, additionally, separated into two groups à 8–14 cows on three farms to investigate different supplementation strategies or differences between animal breeds. Animals were chosen to obtain groups of similar mean days in milk (**DIM**) and mean parity per farm across all sampling phases. Hence, a total of n = 33 animal groups were sampled across both years.

### *Grazing management of study farms*

Dairy cows grazed semi-natural, permanent grasslands with botanically diverse swards, fertilised solely with organic cow manure. The paddock management decisions (i.e., stocking density, daytime of pasture access, and choice of paddocks) recorded by the farm managers are summarised in Table 3. Grazing took place for 4–20 h/d during daytime (n = 169 cows), at night-time (n = 80 cows), or full-time (n = 74 cows). The 1–8 paddocks available to the lactating dairy cows per farm were either grazed continuously or rotated daily. Different combinations of concentrates (mainly cereals), fresh meadow grass, fresh clover-grass swards, grass hay, maize silage, and grass silage were supplemented in the barn. Daily supplementation of concentrate feeds ranged from 0 to 5.6 kg DM/cow which translated to a concentrate inclusion of 0–244 g/kg milk. Total supplement feed (i.e., roughage plus concentrate feeds) ranged from 0 to 18.2 kg DM/cow.

Once per sampling period and farm, the standing pasture herbage on offer was sampled in three or six representative points per paddock, depending on paddock size. Per sampling point, the botanical composition of the vegetation was classified visually according to the share of grasses of the total fresh aboveground herbage biomass. Swards consisting of > 60% grasses were considered as grass-rich swards, whereas those with $\leq$ 45% grasses were classified as herb-rich. Swards with > 45% to $\leq$ 60% of grasses were considered balanced. Per sampling point, aboveground herbage biomass was manually harvested at 3 cm above the ground surface in a 1-$m^2$-plot (0.5 m × 2.0 m), weighed fresh, and processed in the laboratory on the same day or the day after. Daily herbage biomass available to each cow (i.e., herbage allowance; kg DM/cow and d) was estimated by dividing herbage mass (kg DM/ha) by the stocking density on each paddock (n/ha). Mean herbage allowance

**Table 1**
Details related to experimental farms and sampling across four sampling phases [1] on nine commercial, organic dairy cattle farms in South Germany using temperate semi-natural grasslands for grazing.

| | General farm characteristics | | | Distribution of sampling periods | | |
|---|---|---|---|---|---|---|
| Farm | Dairy herd size, n | Cow breed | Annual milk yield, kg/cow | Phase | Calendar week | Sampled cows, n |
| A | 30 | Simmental | 5 698 | 1 | 26 | 19 * |
| | | | | 2 | 36 | 14 * |
| | | | | 3 | 23 | 18 * |
| | | | | 4 | 34 | 16 * |
| B | 80 | Holstein-Friesian | 6 439 | 1 | 19 | 10 |
| | | | | 2 | 34 | 9 |
| | | | | 3 | 19 | 10 |
| | | | | 4 | 32 | 10 |
| C | 40 | Simmental | 7 086 | 3 | 22 | 9 |
| | | | | 4 | 38 | 9 |
| D | 100 | Simmental | 7 430 | 1 | 22 | 10 |
| | | | | 2 | 33 | 10 |
| | | | | 3 | 21 | 28 * |
| | | | | 4 | 37 | 23 * |
| E | 70 | Simmental, Brown Swiss | 7 610 | 3 | 25 | 17 * |
| | | | | 4 | 35 | 17 * |
| F | 40 | Brown Swiss | 5 224 | 1 | 22 | 9 |
| | | | | 2 | 39 | 9 |
| | | | | 3 | 26 | 10 |
| | | | | 4 | 40 | 10 |
| G | 40 | Brown Swiss, Holstein-Friesian | 6 518 | 3 | 29 | 15 |
| | | | | 4 | 37 | 14 |
| H | 40 | Holstein-Friesian | 8 566 | 3 | 23 | 9 |
| | | | | 4 | 40 | 11 |
| I | 60 | Holstein-Friesian | 7 850 | 4 | 31 | 7 |

* Sampled cows further separated into two groups for investigating different supplementation treatments or differences between cow breeds.

[1] Phase 1: May to July 2019; Phase 2: August to October 2019; Phase 3: May to July 2020; Phase 4: August to October 2020.

**Table 2**
Performance and nutrient intake of lactating dairy cows grazing temperate semi-natural grasslands in South Germany, separated by datasets 1 and 2[1].

| | | Dataset 1 | | | | | | Dataset 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | Units | n | Mean | Median | SD | Min | Max | n | Mean | Median | SD | Min | Max |
| BW | kg | 323 | 703 | 691 | 90.8 | 408 | 1 021 | 152 | 689 | 680 | 81.9 | 540 | 1 021 |
| Parity | n | 323 | 3.62 | 3.00 | 2.000 | 1.00 | 11.00 | 152 | 3.74 | 3.00 | 1.917 | 1.00 | 9.00 |
| Milk yield | kg/d | 323 | 23.9 | 23.8 | 5.35 | 9.7 | 39.8 | 152 | 23.2 | 23.0 | 5.53 | 9.7 | 37.2 |
| Milk fat | g/100 g | 323 | 3.93 | 3.86 | 0.544 | 2.37 | 6.04 | 152 | 3.97 | 3.92 | 0.529 | 3.02 | 5.50 |
| Milk protein | g/100 g | 323 | 3.26 | 3.23 | 0.305 | 2.54 | 4.29 | 152 | 3.26 | 3.23 | 0.335 | 2.54 | 4.29 |
| DMI | kg/d | 323 | 21.0 | 21.0 | 3.21 | 12.6 | 29.1 | 152 | 20.7 | 20.9 | 3.23 | 12.6 | 28.6 |
| Pasture DMI | kg/d | 323 | 11.3 | 10.7 | 4.83 | 0.6 | 23.3 | 152 | 10.5 | 9.8 | 4.58 | 0.6 | 23.2 |
| N intake | g/d | 323 | 506 | 504 | 105.8 | 294 | 811 | 152 | 514 | 515 | 111.1 | 296 | 811 |

Abbreviations: DMI = DM intake; Min = minimum; Max = maximum; N = nitrogen.

[1] Dataset 2 was a subsample of dataset 1, because it solely contained observations with urine spot samples.

across all grazed paddocks per farm and period was used for any further analysis.

### *Sampling of supplement feedstuffs*

The individual amounts of concentrate intake via automatic feeders were recorded daily. To determine the amount of forage feedstuffs or partial mixed ration offered and refused by the entire herd of lactating cows, records of the scales built into the forage wagons were used, or the daily mass of offered and refused feed on the feeding banks was weighed manually. Samples of offered and refused supplement feed were collected (∼200 g of fresh matter). Hay and concentrate samples were stored at room temperature, whereas samples of fresh forages and partial mixed rations were frozen immediately after collection (−20 °C).

### *Animal measurements*

All animal procedures were conducted according to the guidelines of the German animal welfare act and were approved by the Institutional Animal Care and Use Committee of the University of Hohenheim. All animals were milked twice daily in the morning and afternoon or had access to an automatic milking system (n = 2 farms). They had free access to drinking water troughs, both in the free-stall barns and on pasture. Parity and DIM of individual animals at the time of sampling were retrieved from official milk reports by the regional association for milk inspection (LKV, Landesverband Baden-Württemberg für Leistungs- und Qualitätsprüfungen in der Tierzucht e.V.). Individual milk yield was measured and sampled once a day from Days 6–11, alternating between morning and afternoon. Milk samples (40 ml) were stored with 150 µL Bronysolv (ANA.LI.TIK Austria, Vienna, Austria) at 4 °C until analysis. The animal's BW was estimated once per period by measuring the heart girth with a tape calibrated for dual-purpose breeds (Animeter, Albert Kerbl GmbH, Buchbach, Germany).

The daily DM intake (**DMI**) of each cow per farm was estimated using a double-marker technique. For this, cows were orally dosed with 24–28 g/d of the external marker $TiO_2$ (Glindemann et al., 2009) for the entire examination period. After morning and evening milking on Days 6–11, faecal grab samples (∼300 g fresh matter) were taken from the animals' rectum, frozen immediately after collection, and stored at −20 °C. On farms with two animal groups

**Table 3**
Descriptive statistics of various grazing management factors determined for grazing-based dairy cattle production systems using temperate semi-natural grasslands in South Germany, separated by datasets 1 and 2[1].

| | | Dataset 1 | | | | | | Dataset 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Grazing management factors | Unit | n | Mean | Median | SD | Min | Max | n | Mean | Median | SD | Min | Max |
| DIM | d | 323 | 146 | 145 | 65.0 | 12 | 412 | 152 | 151 | 158 | 61.3 | 27 | 288 |
| Supplementation strategy | | | | | | | | | | | | | |
| Supplement DMI | kg/d | 33 | 9.68 | 10.53 | 4.881 | 0.51 | 18.18 | 26 | 10.2 | 10.6 | 4.96 | 0.5 | 18.2 |
| Total fresh forage | g/100 g DMI | 33 | 70.0 | 77.6 | 21.95 | 17.6 | 100.0 | 26 | 72.2 | 78.1 | 22.56 | 23.0 | 100.0 |
| Fresh forage | g/100 g supplement DMI | 33 | 29.3 | 0.0 | 34.09 | 0.0 | 100.0 | 26 | 36.2 | 37.4 | 36.20 | 0.0 | 100.0 |
| Grass hay | g/100 g supplement DMI | 33 | 17.0 | 5.1 | 22.10 | 0.0 | 100.0 | 26 | 11.8 | 2.3 | 16.48 | 0.0 | 57.8 |
| Silage[2] | g/100 g supplement DMI | 33 | 23.9 | 0.0 | 33.51 | 0.0 | 100.0 | 26 | 25.5 | 0.0 | 35.48 | 0.0 | 100.0 |
| Concentrates[3] | g/100 g supplement DMI | 33 | 23.9 | 28.3 | 22.39 | 0.0 | 100.0 | 26 | 14.1 | 4.5 | 17.39 | 0.0 | 59.1 |
| Paddock management | | | | | | | | | | | | | |
| CP of pasture | g/100 g DM | 33 | 16.7 | 16.7 | 2.93 | 11.0 | 22.7 | 26 | 17.0 | 16.7 | 3.36 | 11.0 | 22.7 |
| NDF of pasture | g/100 g DM | 33 | 45.2 | 44.6 | 3.75 | 38.0 | 52.0 | 26 | 45.6 | 46.0 | 4.32 | 38.0 | 52.0 |
| ME of pasture | MJ/kg DM | 33 | 9.32 | 9.23 | 0.624 | 8.11 | 10.37 | 26 | 9.27 | 9.23 | 0.634 | 8.11 | 10.26 |
| Stocking density | cows/ha | 33 | 17.2 | 13.2 | 10.83 | 5.0 | 43.7 | 26 | 21.4 | 20.0 | 12.28 | 5.0 | 43.7 |
| Paddock size | ha | 33 | 3.76 | 3.47 | 2.204 | 0.92 | 8.17 | 26 | 3.33 | 3.11 | 2.254 | 0.92 | 8.17 |
| Herbage allowance | kg DM/cow and d | 33 | 29.3 | 16.1 | 24.89 | 6.7 | 94.8 | 26 | 22.5 | 14.2 | 20.88 | 6.7 | 73.2 |
| Herbage mass | kg DM/ha | 33 | 380 | 315 | 283.7 | 84 | 1 558 | 26 | 340 | 292 | 210.0 | 108 | 1 113 |
| Grazing time | h/d | 33 | 10.7 | 10.0 | 5.33 | 3.5 | 20.0 | 26 | 10.1 | 8.0 | 4.82 | 3.5 | 20.0 |
| Botanical composition* | | | | | | | | | | | | | |
| Grass-rich | | 118 | – | – | – | – | – | 59 | – | – | – | – | – |
| Balanced | | 107 | – | – | – | – | – | 42 | – | – | – | – | – |
| Herb-rich | | 98 | – | – | – | – | – | 51 | – | – | – | – | – |
| Daytime of pasture access* | | | | | | | | | | | | | |
| Daytime | | 169 | – | – | – | – | – | 91 | – | – | – | – | – |
| Night-time | | 80 | – | – | – | – | – | 35 | – | – | – | – | – |
| Full-time | | 74 | – | – | – | – | – | 26 | – | – | – | – | – |

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy.

[1] Dataset 2 was a subsample of dataset 1, solely containing records of cows from which urine spot samples were collected.

[2] Silage: grass or maize silage.

[3] Concentrates: different combinations of oat, barley, maize, wheat bran, wheat, triticale, and partly protein concentrates such as peas.

* Categorial factors.

per period, faecal samples were taken only once daily, whereby sampling of each animal group alternated between morning and evening across the six sampling days.

Concomitant to faecal sampling, urine spot samples were collected on six farms from 3 to 10 cows per farm or animal group from Days 6–11 of every period. Urination was stimulated by gentle massage of the perineal area. Samples were collected in plastic buckets, homogenised, and strained to remove any solid impurities. Then, subsamples of $\sim$200 ml were acidified with sulphuric acid (20% v/v) to reduce pH to $< 3$. Three aliquots of $\sim$40 ml of the acidified urine samples were stored at $-20$ °C until analysis.

### *Laboratory analyses*

Milk samples were analysed by the Milchprüfring Baden-Württemberg e.V. (Kirchheim/Teck, Germany) for their fat, protein, and lactose concentrations according to ASU L 01.00-78, 2018-06, as well as milk urea N (**MUN**) according to 05022100.QMD, 2011-03 (Milchprüfring Baden-Württemberg e.V., 2023). For statistical analyses, daily milk yield and composition were averaged per cow and period, whereby records were only considered if samples of one consecutive morning and evening milking were available.

After thawing, faecal samples were homogenised and pooled by cow and period using aliquots of $\sim$15 g fresh matter of each daily sample. The pooled sample ($\sim$200 g of fresh matter) was weighed and then lyophilised for 72 h (LYO GT2 Basis, SRK Systemtechnik GmbH, Riedstadt, Germany). Similarly, urine samples were thawed, pooled by cow by taking 10 mL from each individual sample, and frozen until analysis. Samples of pasture herbage and offered as well as refused supplement feeds were dried at 45 °C for 72 h, except of samples including silages, which were lyophilised. After drying, all samples were weighed for DM determination, and ground through a 1-mm-sieve (Retsch SM 100, Retsch GmbH, Haan, Germany). Then, the entire samples of pasture herbage were pooled by paddock and period. Offered and refused feeds were each pooled by farm and period. For each pooled sample, a subsample of $\sim$50 g dried sample material was taken for laboratory analyses.

All urine, faeces, and feed samples were analysed in duplicate using the official analytical methods in Germany (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2007). Samples of offered and refused supplement feeds, faeces, and herbage were analysed for DM and organic matter (**OM**) (method 3.1 and 8.1, respectively). Concentrations of N in samples of pasture herbage and of offered and refused supplement feed were determined by Dumas combustion (method 4.1.2) using a vario MAX CN analyser (Elementar Analysesysteme GmbH, Langenselbold, Germany). Offered feeds were also analysed for NDF (including residual ash) (method 6.5.1) by an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, US) using sodium sulphite and heat-stable $\alpha$-amylase. The Hohenheim Gas Test was used to estimate metabolisable energy (**ME**) concentrations of offered feedstuffs in triplicate in two independent runs following the procedures described by Menke and Steingass (1988). Specific equations were used for roughage (eq. 16e), concentrate (eq. 14b), and mixed feedstuffs (eq. 12f). Urine and faeces samples were analysed for N by Kjeldahl digestion (method 4.1.1; Vapotest 45 s, C. Gerhardt GmbH & Co. KG, Königswinter, Germany and behrostest K20L, Behr Labor-Technik GmbH, Düsseldorf, Germany). Faecal $TiO_2$ concentrations were determined according to Boguhn et al. (2009) with a modified digestion time of 4 h instead of 40 min to ensure maximum transparency of the digested solution. Concentrations of purine derivatives (**PD**; i.e. allantoin plus uric acid) and creatinine (**C**) in urine were analysed using high-performance reversed-phase liquid chromatography following the procedures by Balcells et al. (1992). The CP concentrations of feed and faecal samples were calculated by multiplying the N concentration by 6.25.

### *Calculation of intake and N excretion and utilisation parameters*

More details on the determination of the DMI of supplements at the herd level and of the DMI of individual animals are described in Perdana-Decker et al. (2023a). In brief, the DMI of supplemental feed consumed in the barn (i.e., supplement DMI) was determined using the records of consumed concentrates by automatic feeding stations, plus the daily measurements of forage feedstuffs or partial mixed ration consumed by the entire herd of lactating cows divided by the number of animals per herd. Daily DMI was calculated from the apparent total tract digestibility of ingested OM based on faecal CP concentration (Lukas et al., 2005), daily faecal OM output as estimated from the daily $TiO_2$ dosage and its concentration in faeces (Glindemann et al., 2009), and from the approximated OM concentration of the total ration. Finally, the DMI of pasture herbage (i.e., pasture DMI) was calculated by subtracting supplement DMI from the total daily DMI of individual cows.

Individual N intake was calculated from the daily supplement DMI plus the animals' individual pasture DMI, each multiplied by the respective N concentrations. Milk N secretion was calculated from daily milk yield and milk N concentration. The latter was obtained by dividing the milk protein contents (g/kg milk) by 6.38 (Gesellschaft für Ernährungsphysiologie (GfE), 2023). Individual faecal N excretion was estimated using the faecal OM excretion by each cow multiplied by the respective N concentration in faecal OM. Urinary N excretion of individual cows was estimated as the difference between N intake and the sum of milk N secretion and faecal N excretion (all in g/d). Milk N secretion and faecal and urinary N excretions were calculated in absolute terms (g/d) and relative to the daily N intake of each animal (g/100 g N intake). In the following, milk N secretion in g/100 g N intake is referred to as MNE. The ratios between PD (mmol/L) and C (g/L) (i.e., PD:C ratio) and between PD and N (g/L) (i.e., PD:N ratio) in urine were considered as indicators for ruminal microbial CP synthesis and the efficiency of dietary N utilisation for ruminal microbial CP synthesis, respectively (Tas and Susenbeth, 2007). Finally, the N:C ratio in urine (both in g/L urine) was considered another indicator for daily urinary N excretion (Chizzotti et al., 2008).

### *Statistical analysis*

The present study comprised a total of 325 individual animal observations. Two different datasets were used to analyse the relationship between 16 grazing management factors (Table 3) and the eight indicators of N utilisation and excretion (Table 4). Dataset 1, comprising all 325 animal-individual observations, was used to analyse three indicators of N utilisation, including milk N secretion, MNE, and MUN concentration, and two indicators of N excretion (relative faecal and urinary N excretions). Dataset 2 only comprised records of cows from which urine samples were collected (n = 152). Hence, this dataset was used to analyse two indicators of N utilisation (ratios of PD:C and PD:N in urine) and one indicator for urinary N excretion (N:C ratio). Both datasets were checked for outliers via their interquartile range, prior to statistical analyses. Two outliers were detected and excluded from dataset 1, because one observation was below and another observation above 1.5 times the interquartile range. For visual inspection of the correlation between grazing management and N-related indicators, a correlation matrix was plotted for each dataset using the R package corrplot.

A variable selection analysis was conducted to identify which grazing management factors were relevant for explaining the variation in N-related indicators. For each N-related indicator separately, backward elimination using a linear mixed-effects model was applied. The N-related indicator served as dependent variable, while the farm, sampling phase nested in farm, and cow nested in farm served as random effects. The random cow effect accounted for the instances when repeated measurements were taken of the same cow over several sampling phases. In total, 16 factors characterising supplementation strategy and paddock management were considered as candidate variables for each backward elimination analysis (Table 3), where the initial model was fitted using the R package lme4. Irrelevant grazing management variables were removed step-by-step based on *P*-values with a selection level of $\alpha = 0.1$, using the R function step by the R package lmerTest (Kuznetsova et al., 2017).

After variable selection via backward elimination, the resulting reduced models were used to identify potential interaction effects. For this, seven predetermined interaction terms were added separately to the selected models. Per N-related indicator, the model with the lowest Akaike Information Criterium among the seven models containing interaction terms was reported, given that the addition of the interaction decreased the Akaike Information Criterium by $\geq 5$. To determine the proportion of the variation explained by the selected fixed effects and random effects, the final models' conditional and marginal $R^2$ were calculated using the r.squaredGLMM function by the R package MuMIn (Bartón, 2022). The conditional and marginal $R^2$ represent the variation explained by the total model and fixed effects, respectively. The difference between conditional and marginal $R^2$ indicates the share of variation explained by the random effects.

Further, to evaluate the model selection stability of the above-mentioned backward elimination analysis, a bootstrap approach was applied (Heinze et al., 2018). Per dependent factor, the backward elimination process was repeated on 1 000 bootstrap resamples drawn using the function bootstrap by the R package sjstats (Lüdecke, 2018). Consequently, the model inclusion frequency for each potential fixed effect, and the frequency of positive and negative signs of the resulting regression coefficients were calculated using the R package bootStepAIC (Rizopoulos, 2022) with slight adaptations to the linear mixed-effects models. All statistical analyses were conducted using R Version 4.2.0 (R Core Team, 2022).

## Results

### *Nitrogen utilisation of lactating dairy cows grazing on semi-natural grassland*

The two datasets captured a diverse set of organic dairy production systems using multispecies, permanent grassland for grazing, comprising 323 animal-individual observations (Tables 2 and 3). This diversity in grazing systems resulted in a wide range of N intake (294–811 g/d), total N excretion (182–697 g/d), and MNE values (11–39 g/100 g N intake) (Table 4). On average (± SD), the ingested N was predominantly excreted via urine (48.9 g/100 g N intake ± 9.77), and to a smaller extend via faeces (26.4 g/100 g N intake ± 4.96). In contrast, MNE averaged 24.7 g/100 g N intake (± 5.91) across all farms, years, and periods. Dataset 2 was a representative subsample of dataset 1 with a similar mean milk production, pasture DMI, and MNE of 23.2 kg/d (± 5.53), 10.5 kg/d (± 4.58), and 23.6 g/100 g N intake (± 6.02), respectively, compared to the values of dataset 1 (23.9 kg/d ± 5.35, 11.3 kg/d ± 4.83, and 24.7 g/100 g N intake ± 5.91).

Daily milk yield correlated moderately with the animals' DIM ($r = -0.55$), their MNE ($r = 0.56$), and relative urinary N excretion

**Table 4**
Descriptive statistics of indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, separated by datasets 1 and 2[1].

| | | Dataset 1 | | | | | | Dataset 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N-related indicators | Unit | n | Mean | Median | SD | Min | Max | n | Mean | Median | SD | Min | Max |
| N utilisation indicators | | | | | | | | | | | | | |
| Milk N secretion | g/d | 323 | 121 | 121 | 24.5 | 58 | 190 | 152 | 117 | 116 | 24.3 | 58 | 176 |
| MNE | g/100 g N intake | 323 | 24.7 | 24.5 | 5.91 | 11.4 | 39.5 | 152 | 23.6 | 22.4 | 6.02 | 11.4 | 39.2 |
| MUN | mg/dL | 323 | 10.1 | 9.2 | 4.27 | 3.3 | 27.0 | 152 | 10.7 | 9.9 | 4.57 | 4.0 | 27.0 |
| PD:C | mmol/g | – | – | – | – | – | – | 152 | 28.2 | 27.3 | 5.41 | 19.7 | 47.8 |
| PD:N | mmol/g | – | – | – | – | – | – | 152 | 3.01 | 2.86 | 1.069 | 1.21 | 7.56 |
| N excretion indicators | | | | | | | | | | | | | |
| Faecal N excretion | g/d | 323 | 131 | 130 | 23.4 | 67 | 184 | 152 | 124 | 123 | 21.7 | 67 | 172 |
| Urinary N excretion | g/d | 323 | 254 | 248 | 93.1 | 79 | 529 | 152 | 273 | 277 | 99.5 | 80 | 529 |
| Total N excretion | g/d | 323 | 385 | 381 | 101.0 | 182 | 697 | 152 | 397 | 393 | 106.9 | 186 | 697 |
| Faecal N excretion | g/100 g N intake | 323 | 26.5 | 26.3 | 4.96 | 15.2 | 37.2 | 152 | 24.9 | 23.7 | 4.97 | 15.2 | 35.2 |
| Urinary N excretion | g/100 g N intake | 323 | 48.9 | 47.8 | 9.77 | 25.6 | 71.3 | 152 | 51.6 | 52.3 | 10.07 | 25.6 | 71.3 |
| N:C | g/g | – | – | – | – | – | – | 152 | 10.6 | 9.9 | 4.42 | 2.7 | 25.7 |

Abbreviations: MNE = milk N use efficiency; MUN = milk urea N; N:C = ratio between urinary N and urinary creatinine concentrations; PD:C = ratio between urinary purine derivates and urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives and urinary N concentrations.

[1] Dataset 2 was a subsample of dataset 1, solely containing records of cows from which urine spot samples were collected.

(g/100 g N intake; $r = -0.41$), as well as with several grazing management factors such as stocking density ($r = -0.48$) or share of fresh forage in supplement DMI ($r = -0.37$; Fig. 1). The pasture DMI correlated strongly with supplement DMI ($r = -0.78$) and daily grazing time ($r = 0.62$), and moderately with the CP concentration of pasture herbage ($r = -0.37$). Only a marginal correlation between pasture DMI and paddock management factors was found, such as with daily herbage allowance on pasture ($r = 0.14$) or stocking density ($r = -0.12$). Furthermore, stocking density correlated with the shares of concentrate ($r = -0.66$) and fresh forage ($r = 0.69$) of supplement DMI. However, no correlation was found between the stocking density and the supplement DMI ($r = -0.09$). There was a strong correlation between MUN concentration and the urinary N:C ($r = 0.87$) or PD:N ratios ($r = -0.81$), and between the urinary N excretion as a share of N intake and the N:C ratio ($r = 0.62$; Fig. 2).

### *Results of bootstrapping procedures*

The animals' DIM was selected in 100% of the bootstrapping resamples to predict milk N secretion, MNE, and relative urinary N excretion, and their regression coefficients indicated an increase in N utilisation and a decrease in urinary N excretion with decreasing DIM (Tables 5 and 6). For all other N-related indicators, DIM was selected with a low frequency ($\leq$ 60%). The supplement DMI and shares of different supplement feedstuffs were frequently (>80%) selected for the majority of N-related indicators, except the share of concentrates in supplement DMI. Increasing daily supplement DMI reduced MNE and increased relative urinary N excretion, MUN concentration, and urinary N:C ratio. The share of fresh forage or hay of supplement DMI was frequently included for multiple N-related indicators (i.e., MNE, relative urinary N excretion, N:C ratio, and MUN). Their respective coefficient signs indicated an increase in N utilisation and a decrease in urinary N excretion with increasing shares of forage and hay in supplement DMI. The MUN concentration, however, increased with increasing shares of fresh forage or hay in supplement DMI, indicating a negative relation between N utilisation and forage and hay supplementation. The share of concentrates in supplement DMI was solely selected with a high frequency (>80%) for the N:C ratio, which decreased with increasing concentrate proportion in supplement DMI.

The ME concentration was included with a selection frequency >85% for all N-related indicators except the PD:C ratio. Greater ME concentrations in pasture herbage increased milk N secretion, MNE, relative faecal N excretion, and the PD:N ratio, and reduced the relative urinary N excretion, MUN concentration, and N:C ratio. The NDF concentration of pasture herbage was positively related to faecal N excretion as share of N intake and PD:N ratio, and negatively related to relative urinary N excretion and MUN concentration. The CP concentration of pasture herbage was selected with a frequency >80% for MNE, relative faecal N excretion, MUN concentration, and the N:C ratio. The respective coefficient signs indicated a decrease in N utilisation and an increase in urinary N excretion with increasing CP concentration of pasture herbage.

None of the paddock management factors had a consistent effect across multiple N-related indicators. Stocking density, daytime of pasture access, and daily grazing time were chosen for several indicators with a frequency >80%, but the distribution of their regression coefficient signs was inconsistent or sometimes even contradictory. Regarding the daytime of pasture access, for instance, full-time and night-time grazing were related to a lower urinary PD:N ratio (i.e., lower efficiency of dietary N use for microbial CP synthesis in the rumen) than daytime grazing. In contrast, MNE was greater and MUN concentration lower when animals grazed full-time than only during daytime.

### *Results of backward elimination*

The range of the conditional $R^2$ across all final models selected by backward elimination (0.74–0.99, Table 7) shows that the predictor variables selected prior to statistical analyses explained most of the variation in the outcome parameters. The random effects explained 5–26% of the variation in the indicators of N utilisation and excretion. Selected fixed effects accounted for the greatest share of variation explained by the selected models with a marginal $R^2$ ranging from 0.40–0.89.

Variable selection by the backward elimination, and the signs of their regression coefficients matched the results of the bootstrapping procedure for the majority of N-related indicators (Tables 8 and 9). Regression coefficients of the models selected by backward elimination indicated a strong effect of full-time grazing compared to daytime grazing on milk N secretion, MUN concentration, and PD:C ratio. Full-time grazing reduced MUN concentration by 31 mg/dL and N:C ratio by 18 g/g, and increased PD:C ratio by 29 mmol/g compared to daytime grazing, indicating greater N utilisation when cows grazed full-time. Likewise, the urinary PD:N ratio was greater for full-time than daytime grazing, which contradicted the negative effect of full-time grazing on the urinary PD:N ratio determined by the bootstrapping procedure.

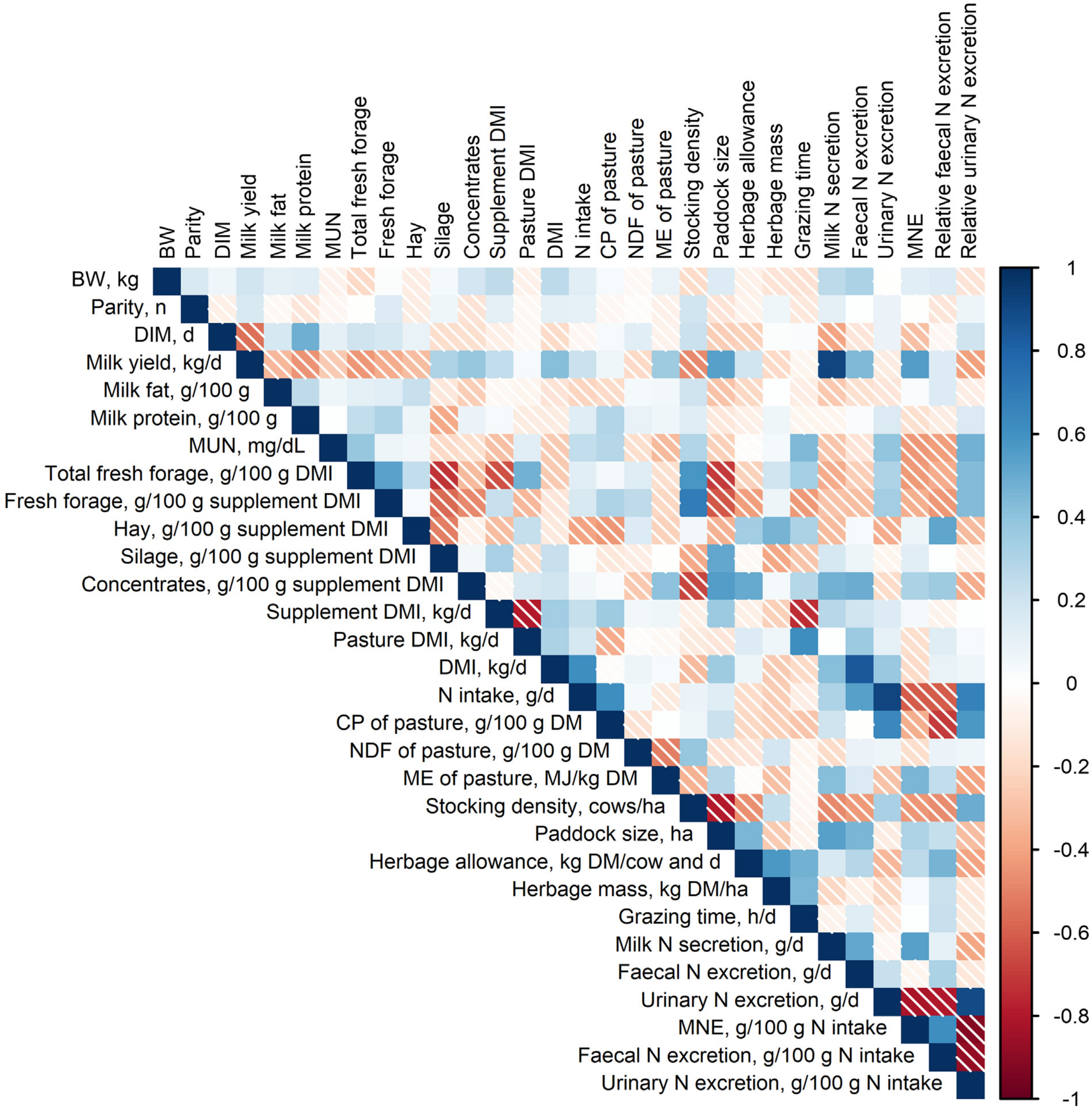

**Fig. 1.** Correlation matrix for dataset 1 (n = 323) with various variables related to milk production, grazing management, and nitrogen (N) utilisation and excretion of lactating dairy cows grazing temperate semi-natural grasslands in South Germany (DIM: days in milk; DMI: DM intake; ME: metabolisable energy; MNE: milk N use efficiency; MUN: milk urea N).

One interaction term was added to each of the mixed models for milk N secretion, relative urinary N excretion, N:C ratio, and PD:N ratio, based on the rule that its addition decreased the models' Akaike Information Criterium by >5, indicating a better fit for the model. The added interaction terms increased the marginal $R^2$ for the N:C and PD:N ratios by 0.07 and 0.25, respectively.

## Discussion

### *Limitations of dataset*

The present study evaluated the N utilisation and excretion of dairy cows grazing semi-natural grasslands Southwest Germany under commercial on-farm conditions. This study region was chosen, because it was representative for regions where permanent, multispecies grasslands constitute the main feed resource which is often the case in semi-mountainous regions in Central Europe (Barrachina et al., 2015). Although only a few farms were included in the present study, they belong to the main types of dairy farms in the study region as determined by Velasco et al. (2021): six farms were grassland-based dairy farms with a great share of their agricultural land being grassland (≥ 61.9%) with relatively low (n = 3) or high annual precipitation (n = 3), three of the study farms represented mixed farming systems with a considerable proportion of their land used for cropping (≥ 32.8%) with variable annual precipitation, and one farm was characteristic of the larger mixed farming systems in the region with a greater grassland and cropping area than the other farm types.

Correlations between several grazing management factors were detected in the present study, such as between the stocking density and the share of concentrates of supplement DMI, which were not necessarily of a causal nature. These correlations should be recognised when interpreting the present study's findings, despite their non-causality as they could not be prevented in our on-farm

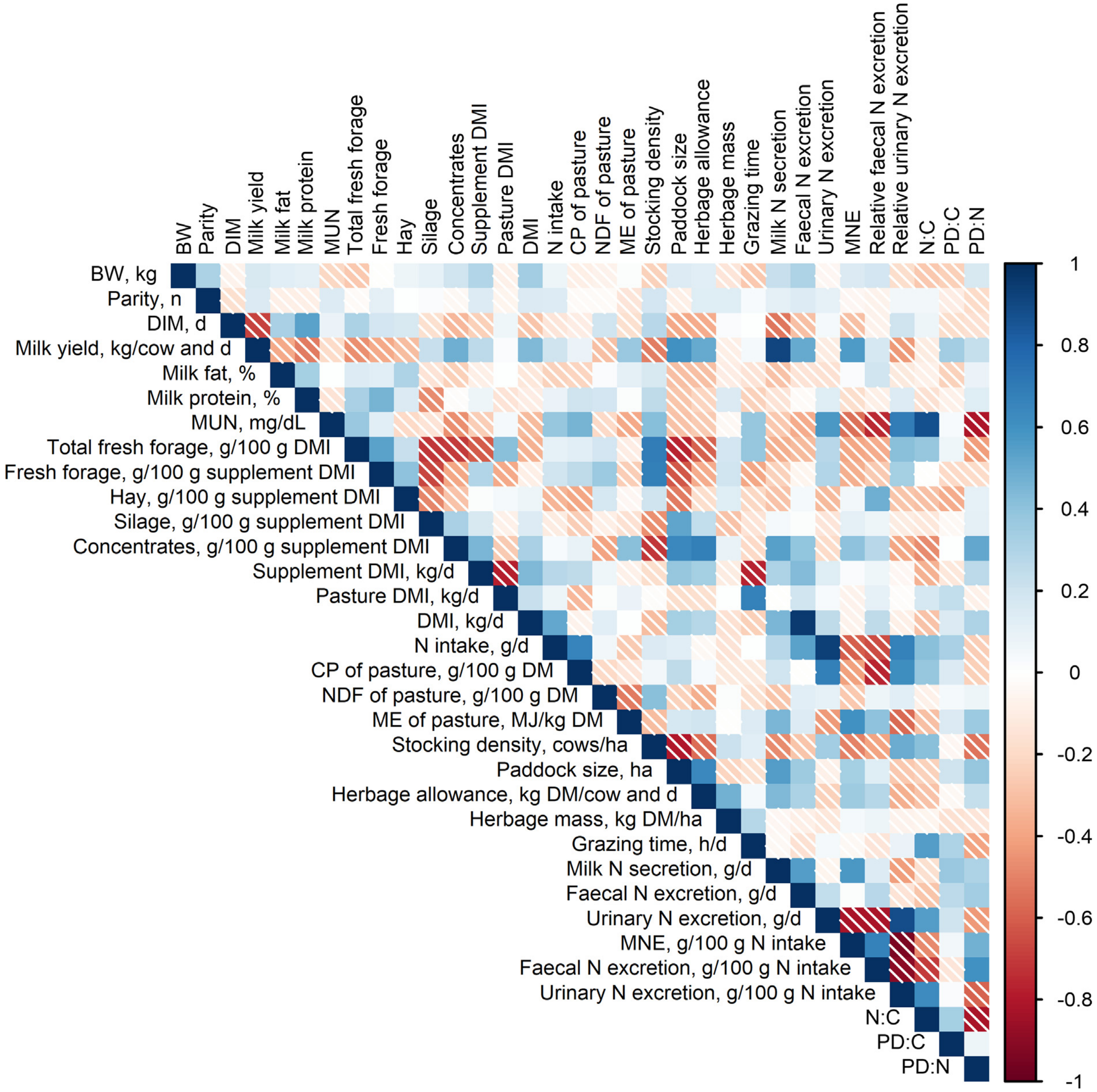

**Fig. 2.** Correlation matrix for dataset 2 (n = 152; i.e., subsample of dataset 1 solely containing records of cows from which urine spot samples were collected) with various variables related to milk production, grazing management, and nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany (DIM = days in milk; DMI = DM intake; ME = metabolisable energy; MNE = milk N use efficiency; MUN = milk urea N; N:C = ratio between urinary N and urinary creatinine concentrations; PD:C = ratio between urinary purine derivates and urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives and urinary N concentrations).

approach. Nevertheless, compared to controlled experiments on research stations testing one or few treatments, such on-farm research allows for analyses of multiple interactions between diverse factors, and thus an understanding of the true effects of grazing management across the diverse production conditions in low-input dairy farms. This approach was, therefore, deliberately chosen to investigate the practical implications of adaptations in grazing management on N utilisation and excretion in low-input, grazing-based dairy farms.

Urinary N excretion was estimated as the difference between the daily N intake and the sum of the daily milk N secretion and faecal N excretion of cows, because urine volume could not be measured. These estimates may be subject to measurement errors in determining N intake and faecal N excretion. The N intake, for instance, was calculated from supplement and pasture DMI and the respective N concentrations of these feedstuffs. The supplement DMI was determined on herd level, which did not capture the variation in N intake from supplement feedstuffs between individual cows. A bias in N intake from pasture herbage is also possible, because pasture herbage samples could not fully reflect the nutritive value of ingested pasture herbage due to the cows' selective grazing behaviour (Schneider et al., 2011). The N:C ratio in urine spot samples, however, served as an independent, measured indicator for daily urinary N excretion and correlated moderately (r = 0.62) with estimated urinary N excretion as proportion of daily N intake. Moreover, the ratios of N:C and PD:N in urine correlated strongly with MUN concentrations (*r* = 0.87 and −0.81, respectively), and the latter is considered a reliable indicator for MNE and urinary N excretion in dairy cows (Spek et al., 2013; Huhtanen et al., 2015). The correlations between the different

**Table 5**

Inclusion frequencies and frequencies of positive and negative coefficient signs of selected mixed regression models using backward elimination in M = 1 000 bootstrap resamples to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 1 (n = 323).

| | | Milk N secretion, g/d | | | MNE, g/100 g N intake | | | Faecal N excretion, g/100 g N intake | | | Urinary N excretion, g/100 g N intake | | | MUN, mg/dL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sign, % | | | Sign, % | | | Sign, % | | | Sign, % | | | Sign, % | |
| Predictor variable | Unit | Selection, % | + | − | Selection, % | + | − | Selection, % | + | − | Selection, % | + | − | Selection, % | + | − |
| DIM | d | 100 | 0 | 100 | 100 | 0 | 100 | 14 | 38 | 62 | 100 | 100 | 0 | 30 | 22 | 78 |
| Supplement DMI | kg/d | 95 | 100 | 0 | 100 | 0 | 100 | 22 | 38 | 62 | 93 | 98 | 2 | 93 | 99 | 1 |
| Total fresh forage | g/100 g DMI | 81 | 100 | 0 | 100 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 0 | 36 | 16 | 84 |
| Fresh forage | g/100 g supplement DMI | 77 | 1 | 99 | 100 | 100 | 0 | 50 | 92 | 8 | 100 | 0 | 100 | 91 | 99 | 1 |
| Hay | g/100 g supplement DMI | 59 | 3 | 97 | 91 | 100 | 0 | 69 | 48 | 52 | 88 | 0 | 100 | 93 | 99 | 1 |
| Concentrates | g/100 g supplement DMI | 55 | 93 | 7 | 66 | 1 | 99 | 45 | 56 | 44 | 57 | 99 | 1 | 79 | 95 | 5 |
| CP of pasture | g/100 g DM | 50 | 84 | 16 | 84 | 0 | 100 | 88 | 0 | 100 | 66 | 99 | 1 | 99 | 100 | 0 |
| NDF of pasture | g/100 g DM | 75 | 98 | 2 | 53 | 91 | 9 | 91 | 100 | 0 | 92 | 0 | 100 | 95 | 0 | 100 |
| ME of pasture | MJ/kg DM | 92 | 100 | 0 | 100 | 100 | 0 | 85 | 100 | 0 | 100 | 0 | 100 | 100 | 0 | 100 |
| Botanical class (basis: Balanced) | | 62 | | | 52 | | | 71 | | | 85 | | | 92 | | |
| Grass-rich | | | 65 | 35 | | 87 | 13 | | 41 | 59 | | 0 | 100 | | 71 | 29 |
| Herb-rich | | | 89 | 11 | | 28 | 72 | | 0 | 100 | | 96 | 4 | | 0 | 100 |
| Stocking density | n/ha | 57 | 2 | 98 | 53 | 24 | 76 | 48 | 15 | 85 | 94 | 100 | 0 | 92 | 3 | 97 |
| Paddock size | ha | 44 | 55 | 45 | 64 | 88 | 12 | 17 | 1 | 99 | 42 | 85 | 15 | 84 | 2 | 98 |
| Herbage allowance | kg DM/cow and d | 75 | 0 | 100 | 73 | 1 | 99 | 59 | 100 | 0 | 49 | 100 | 0 | 68 | 3 | 97 |
| Herbage mass | kg DM/ha | 53 | 91 | 9 | 27 | 82 | 18 | 43 | 0 | 100 | 5 | 34 | 66 | 55 | 62 | 38 |
| Daytime of grazing (basis: Daytime) | | 71 | | | 100 | | | 63 | | | 100 | | | 93 | | |
| Full-time | | | 96 | 4 | | 94 | 6 | | 75 | 25 | | 69 | 31 | | 1 | 99 |
| Night-time | | | 84 | 16 | | 6 | 94 | | 2 | 98 | | 100 | 0 | | 1 | 99 |
| Grazing duration | h/d | 52 | 32 | 68 | 41 | 12 | 88 | 37 | 92 | 8 | 69 | 0 | 100 | 99 | 100 | 0 |

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; MNE = milk N use efficiency; MUN = milk urea nitrogen.

**Table 6**

Inclusion frequencies and frequencies of positive and negative coefficient signs of selected mixed regression models using backward elimination in M = 1 000 bootstrap resamples to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 2, which included indicators based on urine spot sampling (n = 152).

| | | N:C, g/g | | | PD:C, mmol/g | | | PD:N,mmol/g | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sign, % | | | Sign, % | | | Sign, % | |
| Predictor variable | Unit | Selection, % | + | − | Selection, % | + | − | Selection, % | + | − |
| DIM | d | 41 | 43 | 57 | 54 | 21 | 79 | 60 | 35 | 65 |
| Supplement DMI | kg/d | 93 | 96 | 4 | 49 | 51 | 49 | 57 | 33 | 67 |
| Total fresh forage | g/100 g DMI | 47 | 25 | 75 | 48 | 71 | 29 | 63 | 58 | 42 |
| Fresh forage | g/100 g supplement DM | 53 | 18 | 82 | 56 | 16 | 84 | 56 | 37 | 63 |
| Grass hay | g/100 g supplement DM | 39 | 13 | 87 | 58 | 8 | 92 | 56 | 28 | 72 |
| Concentrates | g/100 g supplement DM | 86 | 1 | 99 | 54 | 93 | 7 | 37 | 89 | 11 |
| CP of pasture | g/100 g DM | 92 | 100 | 0 | 63 | 9 | 91 | 38 | 31 | 69 |
| NDF of pasture | g/100 g DM | 75 | 6 | 94 | 64 | 40 | 60 | 88 | 97 | 3 |
| ME of pasture | MJ/kg DM | 89 | 1 | 99 | 50 | 89 | 11 | 81 | 97 | 3 |
| Botanical class (Basis: Balanced) | | 83 | | | 99 | | | 62 | | |
| Grass-rich | | | 45 | 55 | | 92 | 8 | | 70 | 30 |
| Herb-rich | | | 9 | 91 | | 99 | 1 | | 71 | 29 |
| Stocking density | n/ha | 51 | 28 | 72 | 86 | 98 | 2 | 77 | 30 | 70 |
| Paddock size | ha | 53 | 25 | 75 | 78 | 90 | 10 | 73 | 35 | 65 |
| Herbage allowance | kg DM/cow and d | 77 | 4 | 96 | 62 | 9 | 91 | 63 | 66 | 34 |
| Herbage mass | kg DM/ha | 68 | 85 | 15 | 48 | 69 | 31 | 66 | 22 | 78 |
| Daytime of grazing (Basis: Daytime) | | 65 | | | 98 | | | 91 | | |
| Full-time | | | 23 | 77 | | 100 | 0 | | 39 | 61 |
| Night-time | | | 30 | 70 | | 98 | 2 | | 23 | 77 |
| Grazing duration | h/d | 89 | 98 | 2 | 85 | 1 | 99 | 50 | 34 | 66 |

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; N:C = ratio between urinary N to urinary creatinine concentrations; PD:C = ratio between urinary purine derivates to urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives to urinary N concentrations.

**Table 7**
Partitioning of the $R^2$ [1] for the linear mixed models determined via backward elimination to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany.

| Partitioning of $R^2$ | Milk N secretion | MNE | Faecal N excretion | Urinary N excretion | MUN | N:C | PD:C | PD:N |
|---|---|---|---|---|---|---|---|---|
| Marginal $R^2$ | 0.54 | 0.67 | 0.83 | 0.84 | 0.40 | 0.89 | 0.52 | 0.80 |
| Conditional $R^2$ | 0.79 | 0.74 | 0.99 | 0.90 | 0.98 | 0.92 | 0.78 | 0.92 |
| Random $R^2$ | 0.25 | 0.07 | 0.16 | 0.06 | 0.58 | 0.03 | 0.27 | 0.12 |

Abbreviations: MNE = milk N use efficiency; MUN = milk urea N; N:C = ratio between urinary N to urinary creatinine concentrations; PD:C = ratio between urinary purine derivates to urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives to urinary N concentration.

[1] $R^2$: partitioned into conditional $R^2$ i.e., variation explained by total statistical model, marginal $R^2$ i.e., variation explained by fixed effects, and random $R^2$ i.e., variation explained by random effects.

**Table 8**
Results of backward elimination with linear mixed models to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 1 (n = 323).

| Predictor variables | Units | Regression coefficients and significances[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Milk N secretion, g/d | | MNE, g/100 g N intake | | Faecal N excretion, g/100 g N intake | | Urinary N excretion, g/100 g N intake | | MUN, mg/dL | |
| Intercept | | −49.86 | | 18.75 | | 14.38 | | 71.32 | | 4.23 | |
| DIM | d | −0.10 | *** | −0.02 | *** | – | | 0.02 | *** | – | |
| Supplement DMI | kg/d | 3.23 | *** | −1.65 | *** | – | | 1.78 | *** | 0.61 | *** |
| Total fresh forage | g/100 g DMI | 0.52 | ** | −0.49 | *** | −0.11 | *** | 0.62 | *** | – | |
| Fresh forage | g/100 g supplement DMI | −0.17 | NS | 0.22 | *** | 0.05 | *** | −0.28 | *** | 0.06 | *** |
| Grass hay | g/100 g supplement DMI | −0.12 | NS | 0.04 | *** | 0.04 | *** | −0.05 | * | 0.06 | *** |
| Concentrates | g/100 g supplement DMI | 0.93 | ** | −0.06 | *** | 0.03 | ** | −4.52 | ** | 0.03 | *** |
| CP of pasture | g/100 g DM | 0.19 | NS | – | | −0.94 | **** | 0.90 | *** | 1.00 | *** |
| NDF of pasture | g/100 g DM | 1.04 | * | 0.29 | *** | 0.30 | * | −0.60 | *** | −0.21 | *** |
| ME of pasture | MJ/kg DM | 6.64 | * | 4.59 | *** | 1.96 | * | −7.45 | *** | −2.11 | *** |
| Botanical class (Basis: Balanced) | | – | | | *** | – | | | * | | *** |
| Grass-rich | | | | 1.61 | | | | −2.91 | | 0.39 | |
| Herb-rich | | | | −0.50 | | | | 0.61 | | −3.38 | |
| Stocking density | n/ha | −0.61 | . | −01.7 | *** | −0.14 | ** | 0.31 | *** | −0.56 | *** |
| Paddock size | ha | – | | – | | – | | – | | −1.09 | *** |
| Herbage allowance | kg DM/cow and d | −0.23 | ** | – | | – | | – | | −0.03 | ** |
| Herbage mass | kg DM/ha | – | | – | | – | | – | | – | |
| Daytime of grazing (Basis: daytime) | | | NS | | *** | – | | | * | | *** |
| Full-time | | 13.47 | | 2.39 | | | | −4.25 | | −30.52 | |
| Night-time | | −1.49 | | −2.23 | | | | 1.66 | | −5.89 | |
| Grazing duration | h/d | 1.54 | NS | – | | – | | – | | 3.05 | *** |
| Grazing duration * concentrates | h/d * g/100 g supplement DMI | −0.04 | ** | – | | – | | – | | – | |
| Concentrates * hay | g/100 g supplement DMI * g/100 g supplement DMI | – | | – | | – | | −0.003 | ** | – | |

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; MNE = milk N use efficiency; MUN = milk urea N.

[1] Significance codes: '***': $P$-value ≤ 0.001, '**': $P$-value ≤ 0.01, '*': $P$-value ≤ 0.05, NS: not significant.

kinds of independently measured N-related indicators, thus, illustrate the robustness and suitability of the chosen N-related indicators to reflect intra-animal variation in N utilisation and excretion of dairy cows.

### *Nitrogen utilisation and excretion of lactating dairy cows grazing temperate, semi-natural grasslands*

It was hypothesised that the limited supplementation with energy-rich concentrates coupled with the supplementation of freshly cut meadow grass or grass-clover mixtures would increase the imbalance between the supply of dietary CP and energy, and thus, reduce N utilisation and increase N excretion in the investigated grazing systems when compared to high-input grazing systems. This hypothesis, however, was not confirmed. Mean MNE (± one SD) of dairy cows averaged 24.7 g/100 g N intake (± 5.91) in the present study. This MNE is similar to or even greater than the reported MNE for grazing dairy cows in North Germany (Löw et al., 2020: 22–25 g/100 g N intake) or for Irish dairy cows grazing high-quality pastures (Doran et al., 2022: 12–25 g/100 g N intake). Urinary N excretion of cows (48.9 g/100 g N intake; ± 9.77) was lower than reported for dairy cattle grazing high-quality ryegrass-clover pastures in Ireland (Doran et al., 2022: 56.8–57.8 g/100 g N intake) or New Zealand (Totty et al., 2013: 64.3–74.0 g/100 g N intake), also indicating lower urinary N losses in the low-input grazing systems of the present study. In temperate high-input grazing systems, dairy cows use high-quality pastures with CP concentrations of up to 30 g/100 g DM that provide an excess of rumen-degradable CP and metabolisable protein relative to the requirements of rumen microbes and their host animals (Waghorn and Clark, 2004), which largely explains the low N utilisation in such grazing systems (Totty et al., 2013). In contrast, cows in the present study grazed multispecies, permanent grasslands with a lower CP concentration of herbage across the entire grazing season (16.7 g/100 g DM ± 2.93). Hence, the lower N intake in the present study (506 g/d ± 105.8) compared to that of grazing cows in New Zealand (Totty et al., 2013: 551–610 g/d) or Ireland (Doran et al., 2022: 546 and 568 g/d) to some degree explains the greater N utilisation and lower urinary N excretion observed here than reported for high-input grazing systems (Castillo et al., 2000).

However, MNE was lower than that measured in solely stall-fed dairy cattle determined in meta-analyses conducted by Spek et al. (2013) for Northwest European dairy farms (27 g/100 g N intake)

**Table 9**
Results of backward elimination with linear mixed models to identify significant predictor variables for indicators of nitrogen (N) utilisation and excretion determined in lactating dairy cows grazing temperate semi-natural grasslands in South Germany, using dataset 2, which included indicators based on urine spot sampling (n = 152).

| | | Regression coefficients and significances[1] | | | | | |
|---|---|---|---|---|---|---|---|
| Predictor variable | Unit | N:C, g/g | | PD:C, mmol/g | | PD:N, mmol/g | |
| Intercept | | 30.06 | | −15.43 | | −5.81 | |
| DIM | d | – | | −0.01 | * | 0.00 | ** |
| Supplement DMI | kg/d | 1.02 | *** | 0.74 | ** | −0.12 | *** |
| Total fresh forage | g/100 g DMI | – | | 0.18 | ** | – | |
| Fresh forage | g/100 g supplement DMI | −0.03 | * | −0.15 | *** | 0.00 | * |
| Grass hay | g/100 g supplement DMI | – | | −0.10 | *** | 0.02 | *** |
| Concentrates | g/100 g supplement DMI | −0.14 | *** | – | | 0.04 | *** |
| CP of pasture | g/100 g DM | 0.27 | ** | – | | – | |
| NDF of pasture | g/100 g DM | −0.44 | *** | 0.32 | *** | 0.11 | *** |
| ME of pasture | MJ/kg DM | −2.93 | *** | 2.22 | *** | 0.75 | *** |
| Botanical class (Basis: Balanced) | | | *** | | *** | | *** |
| Grass-rich | | −1.06 | | 3.44 | | 0.54 | |
| Herb-rich | | −3.55 | | 5.95 | | 0.65 | |
| Stocking density | n/ha | −0.14 | * | 0.29 | ** | – | |
| Paddock size | ha | – | | – | | – | |
| Herbage allowance | kg DM/cow and d | −0.17 | *** | – | | – | |
| Herbage mass | kg DM/ha | 0.01 | *** | – | | – | |
| Daytime of grazing(Basis: Daytime) | | | *** | | *** | | *** |
| Full-time | | −18.14 | | 29.14 | | 2.37 | |
| Night-time | | −5.31 | | 7.47 | | 0.44 | |
| Grazing duration | h/d | 2.55 | *** | −1.97 | *** | −0.31 | *** |
| Concentrates * fresh forage | g/100 g supplement DMI * g/100 g supplement DMI | −0.03 | *** | – | | | |
| Concentrates * hay | g/100 g supplement DMI * g/100 g supplement DMI | – | | – | | 0.00 | *** |

Abbreviations: DIM = days in milk; DMI = DM intake; ME = metabolisable energy; N:C = ratio between urinary N to urinary creatinine concentrations; PD:C = ratio between urinary purine derivates to urinary creatinine concentrations; PD:N = ratio between urinary purine derivatives to urinary N concentrations.

[1] Significance codes: '***': *P*-value ≤ 0.001, '**': *P*-value ≤ 0.01, '*': *P*-value ≤ 0.05, NS: not significant.

and by Schuba et al. (2017) using a global dataset (29 g/100 g N intake). Nevertheless, the lower mean MUN concentration in the present dataset (10.1 mg/dL ± 4.23) compared to values determined by Spek et al. (2013: 12.5 mg/dL ± 5.07) also indicate that relative urea N losses may not be substantially greater in low-input grazing systems than in stall-based feeding systems.

The present dataset further showed a large range in the N-related indicators despite the strong focus on organic dairy farms located in South Germany using temperate semi-natural grasslands for grazing. For instance, MUN concentrations averaged per farm, period, and animal group ranged between 5.0 and 22.5 mg/dL. The lower observed MNE and greater urinary N excretion compared to observations from stall-fed dairy cattle systems and the large range in N-related indicators highlight that, on the one hand, there were periods within farms with a substantially greater risk for N losses through leaching and volatilisation on pastures than under confinement conditions. On the other hand, among the investigated grazing strategies, there were some which achieved levels of N excretion and MNE comparable with those of stall-fed feeding-systems. The management factors explaining these differences were, thus, analysed via a bootstrapping procedure and backward elimination.

### *Effect of performance level*

The MNE and urinary N excretion were calculated from daily milk N secretion. Hence, milk yield was not considered in the bootstrapping analysis and backward elimination for any of the eight indicators of N utilisation and excretion to avoid artifactual correlations. However, there is a close link between nutrient use efficiency and performance level, as seen in the moderate correlation between milk yield and MNE ($r = 0.56$). Therefore, DIM was included to correct the effect of milk yield on the indicators of N utilisation and excretions. As expected, DIM was selected with high frequency for all indicators related to milk yield (i.e., milk N secretion, MNE, relative urinary N excretion) but with low frequency for indicators which were independently determined from milk performance (i.e., ratios of N:C, PD:N, PD:C), suggesting that milk yield played a minor role in explaining variation in microbial CP synthesis and total urinary N excretion. Lastly, it cannot be determined whether DIM significantly affected milk N secretion, MNE, and relative urinary N excretion, because DIM was representative of the animal's productivity or because DIM correlated with milk yield (i.e., represented an artifactual effect).

### *Random effect of sampling phase*

Data collection was repeated twice per year and farm to capture different years and pasture conditions at different times of the year, which were considered to differ with regard to availability, botanical composition, and nutritional quality of pasture herbage and forage supplements (Waghorn and Clark, 2004). During the backward elimination procedure, seasonal and annual differences that were not captured by selected grazing management factors were considered using the random effect of the sampling phase. This random effect only played a role in the mixed models for the relative faecal and urinary N excretions (i.e., no variance estimates were provided for the remaining N-related indicators). However, solely $R^2$ values of 0.06 and 0.16 were attributable to the random effects in the backward selected models for relative urinary and faecal N excretion, respectively. Hence, most variation in relative N excretion was explained by the selected grazing management factors, such as the nutritional quality of pasture herbage, as demonstrated by the marginal $R^2$ of 0.84 and 0.83 for relative urinary and faecal N excretion, respectively.

### *Effect of the nutritional quality of pasture herbage*

Increases in ME and NDF concentrations of pasture herbage were related to increased relative faecal N excretion and MNE, and reduced relative urinary N excretion. The positive effect of ME on N utilisation and simultaneous reduction of urinary N excretion was expected from the more synchronised CP-to-energy ratio in pasture herbage leading to a lower ruminal N balance (Castillo et al., 2000). The CP concentration of pasture herbage, on the other hand, was chosen with high frequency for MUN concentration and urinary N:C ratio, eliciting that it was a key driver for excessive N intake, leading to inefficient ruminal N utilisation and increased milk and urinary urea N excretion (Hoekstra et al., 2007). The nutritional quality of pasture herbage thus had a major influence on the chosen N-related indicators, although supplement DMI had a substantial share of the total DMI (46 g/100 g DMI) of dairy cows grazing semi-natural grasslands.

### *Effect of the amount and type of supplementation*

Results of backward elimination and bootstrapping indicated that the type and amount of supplementation were decisive for N utilisation and excretion. Nitrogen utilisation increased (e.g., MNE) and daily relative urinary N excretion decreased with increasing proportion of fresh forage or grass hay of supplement DMI. At the same time, increases in total supplement DMI had the opposite effect (i.e., reduced N utilisation and increased urinary N excretion). Hence, increasing shares of forages and/or hay in supplement DMI solely increased N utilisation at moderate levels of supplement DMI (i.e., if it did not substitute pasture DMI substantially). These findings support the hypothesis that substitution of full-time grazing with part-time grazing combined with moderate supplementation with more mature forages lower in CP concentration (14.2 g/100 g DM ± 4.37) than young, grazed herbage (16.7 g/100 g DM ± 2.93) would increase dietary N utilisation and thus decrease urinary N excretion of grazing dairy cows.

Concentrate supplementation mainly consisted of energy-rich ingredients (i.e., cereal grains), with mean ME and CP concentrations of 12.1 MJ/kg DM (± 0.67) and 11.7 g/100 g DM (± 2.29), respectively. It was expected that such supplementation with energy-rich concentrates would increase N utilisation and decrease urinary N excretion of grazing cows by supplying readily fermentable carbohydrates in addition to the CP excess from pasture herbage for more efficient microbial CP synthesis. The share of concentrates in supplement DMI was solely chosen for the N-related indicators determined in urine (i.e., dataset 2), and in interaction with the hay and fresh forage share in supplements. Daily urinary N excretion (i.e., N:C) declined and dietary N utilisation for rumen microbial CP synthesis (i.e., PD:C and PD:N) increased with increasing concentrate share of supplement DMI. Consequently, increases in concentrate share in supplement DMI improved the CP-to-energy ratio (i.e., ruminal N balance). However, the improved ruminal N balance did not translate into greater N utilisation for milk protein synthesis (i.e., greater MNE), likely because utilisable protein was above the animal's requirements. The negative interaction coefficients indicated that the concentrate effect was diminished by increasing shares of fresh forage or hay in supplements.

### *Effect of paddock management factors*

In general, paddock management factors were chosen with the lowest frequency in the bootstrapping analysis, and factors chosen with higher frequency showed an inconsistent distribution of their regression coefficient signs. The herbage allowance and stocking density play an important role in determining pasture DMI in high-input grazing systems (e.g., McCarthy et al., 2011; Pérez-Prieto and Delagarde, 2013). In the present study, neither herbage allowance ($r = -0.09$) nor stocking density ($r = -0.09$) correlated notably with pasture DMI, and they did not explain the variation in the observed N-related indicators. The low correlation between these paddock management variables and pasture DMI may be attributed to the substantial supplementation in the barn, substituting pasture DMI and lowering the influence of changes in paddock management on pasture DMI, and, therefore, N utilisation and excretion.

Several indicators demonstrated a greater N utilisation and lower urinary N excretion when animals grazed full-time compared to grazing during the day. This is in contrast to our expectation that N utilisation would be greater with part-time compared to full-time grazing but supports the earlier finding that N utilisation increased, and urinary N excretion decreased with a reduction in supplement DMI (e.g., as a consequence of longer daily access to pasture). In the present study, cows with full-time access to pasture were still supplemented in the barn after each milking time (3.9 kg DM/d), mainly with concentrates (41 g/100 g supplement DMI) and grass hay (28 g/100 g supplement DMI). Apart from the supplementation, no other management factor explained the greater N utilisation and lower N excretion of cows grazing full-time. For instance, they received less concentrates (2.2 kg DM/d) compared to animals grazing during the day (3.0 kg DM/d), had a similar milk yield (24.1 kg/d) than cows with pasture access during daytime (24.4 kg/d), and the CP concentration of their diet (15.1 g/100 g DM) did not differ from that of diets of the daytime group (15.7 g/100 g DM). Although the distribution of cows between the groups grazing during the day (n = 169), night (n = 80), and full-time (n = 74) was unbalanced, these findings indicate that there were farms making great use of their available pasture resource by full-time grazing and moderate supplementation in the barn without excessive urinary N excretion or hampering milk protein yield.

Finally, our results indicate that individual paddock management factors such as botanical composition, herbage allowance, or daytime of grazing, which have been shown to improve availability and nutritional values of pasture herbage, or feed intake, performance, and nutrient use of grazing livestock in controlled experiments, may not have any effect under practical farming conditions. Instead, they may only effectively influence forage intake and use as well as N utilisation and excretion of grazing cows when combined with other grazing management factors (e.g., supplementation strategy).

## Conclusion

The N utilisation and excretion by lactating dairy cows vary greatly across diverse organic dairy farms in South Germany using temperate semi-natural grasslands for grazing. On average, observed MNE is greater than in high-input grazing systems but lower than in stall-fed cows receiving balanced diets. For the investigated farming conditions, the supplementation strategy has the greatest potential for manipulating N utilisation and excretion. Increasing shares of fresh forages as well as of hay in supplement DMI increases N utilisation and decreases urinary N excretion, while increasing shares of concentrates in supplement DMI are related to lower N losses via urine. Hence, full-time grazing combined with moderate supplementation in the barn is a viable option for low-input, grazing-based dairy operations with moderate levels of N losses. Yet, nutritional value of pasture herbage is a key driver for milk protein production and N excretions even at substantial supplementation, emphasising the need for real-time applications to quantify the nutritional quality of pasture herbage to enable adequate supplementation in the barn. The present study was based on on-farm trials which allowed for the analysis of interactions between diverse factors, and thus an understanding of the true effects of grazing management across the diverse production conditions in low-input dairy farms.

## Ethics approval

The study was conducted according to the guidelines of the German animal welfare act and was approved by the Institutional Animal Care and Use Committee of the University of Hohenheim.

## Data and model availability statement

The data were not deposited in an official repository. The data that support the study findings are available from the corresponding author upon request.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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## Declaration of interest

None.

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