Fingerprinting Pasture Phenolics

Aspects of Biodiversity, Animal Health, and Agricultural Practices

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Introduction

Methane emissions, antimicrobial resistance, anthelmintic resistance, loss of biodiversity, animal health and well-being; the list of **veterinary** and **agroecological challenges** is long. In human nutrition, **phenolics** have been well known for their potential health-promoting abilities. In recent years they've also gained the attention of the agricultural and veterinary sector due to their **potential** to tackle these challenges (Makkar et al (2007)). For **grazing animals**, phenolics could be easily and cost-effectively accessed through **pasture**.

Aim of Pilot Study:

What phenolics are present in different pasture types?

- Gain a better understanding of the phenolic profile of three distinct mixed swards regarding potential health-promoting compounds.
- Evaluate the impact of seasonal and agricultural practices (fertiliser application) on phenolic concentrations.
- Identify botanical functional groups affecting the phenolic profile.

Methods

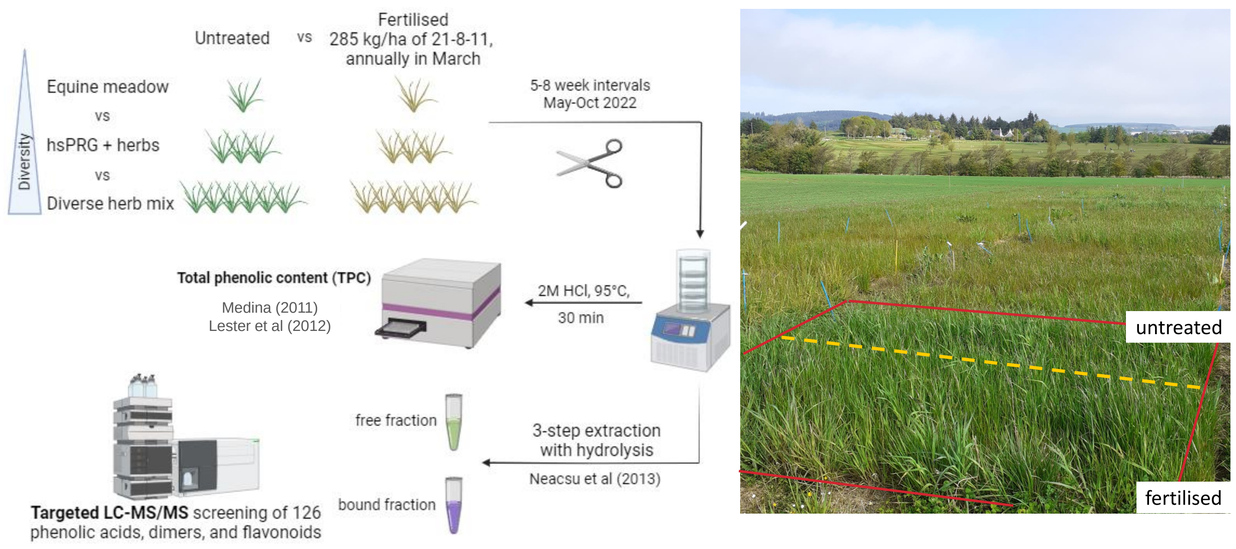

Figure 1. Sample preparation, 3-step phenolic extraction for targeted LC-MS/MS analysis and photometric analysis of total phenolic content (TPC). (created in BioRender)

Figure 2. Experimental site at SRUC, Aberdeen (57.180392° N, 2.221272° W) showing fertilised and untreated plots.

Species composition of grasses, legumes, and herbs differed across the three seed mixtures, with diversity lowest in the equine meadow. Biodiversity increased progressively from the high-sugar perennial ryegrass (hsPRG) to the diverse herb mixture.

Results

TPC was highest in the species-rich sward (z=3.55, p=0.006). LC-MS/MS analysis identified chlorogenic acid, ferulic acid, kaempferol, p-coumaric acid, rutin, ethyl ferulate, and quercetin as the most abundant metabolites across the seed-plot mixes.

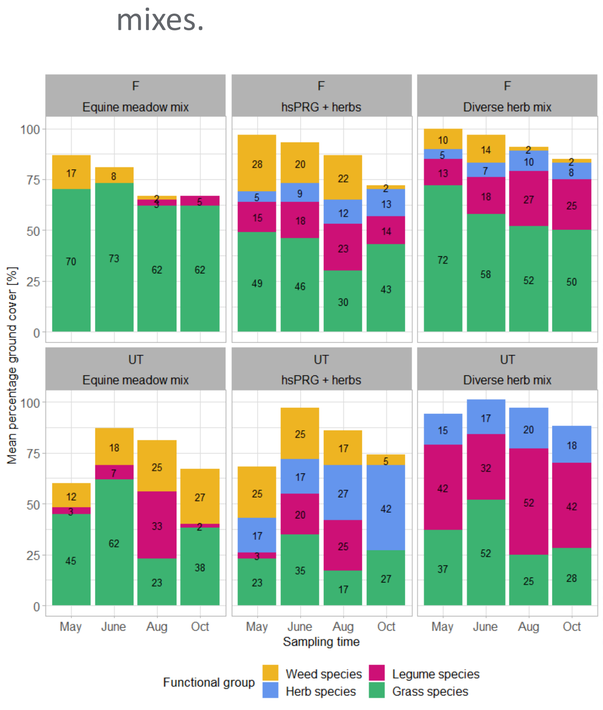

Figure 3. Ground cover estimation of functional groups in fertilised (F) and untreated (UT) plots.

Significant differences in the abundance of functional plant groups were found across the three plots (Figure 3). Species-rich diverse herb mix out-competed weed infestation while equine meadow and hsPRG mix had a higher percentage of weeds and bare ground.

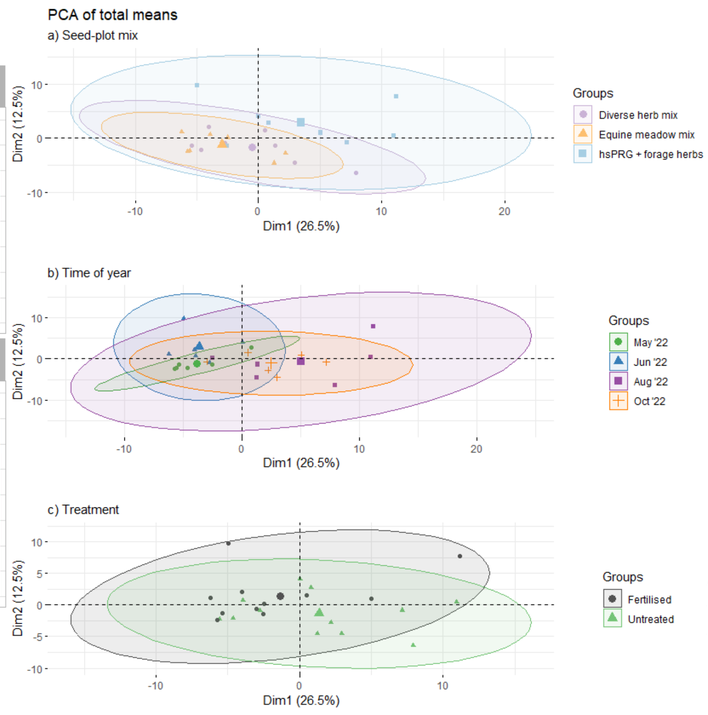

Figure 4. Principal Component Analysis (PCA) of phenolic content (mg/kg DM (dry matter)) measured by LC-MS/MS scaled to unit variance.

The PCA showed: Overlapping clusters of seed-plot mix (Figure 5, a), an increase of molecule diversity in samples collected in August (b), and no differentiation between treatment groups (c).

Figure 5. Total amount of targeted phenolics (sum of individual molecules measured by LC-MS/MS) expressed as g/kg DM and kg/ha DM.

The amount of targeted phenolics in the diverse herb mix was significantly lower than the fertilised equine meadow mix (Z= -2.16, p=0.046) as well as the fertilised (Z=-2.55, p=0.032) and untreated hsPRG + herbs mix (Z=-2.65, p=0.024) per g/kg DM (Figure 4, a). The total amount/ha DM in May was significantly affected by the fertiliser application and showed large seasonal variation with increases until June, followed by a decrease across all seed-plot mixes regardless of treatment (b).

Conclusions

First-time application (to our knowledge) of a 3-step hydrolysis extraction to quantify free and bound metabolites in pasture resuted in the successful detection of 101/126 targeted metabolites:

- Whilst **species-poor mixtures** showed **higher total amount** of targeted metabolites per g/kg DM, s**pecies-rich** diverse herb mix had **higher molecule diversity** (LC-MS/MS) and **higher TPC** (spectrophotometric)
- **Seasonal variation** with increased availability (kg/ha DM) in June
- **No effect of fertiliser** application on total amount of targeted phenolics (**g/kg DM**) but **impact total availability (kg/ha DM)** in May only

The pilot study highlights the potential of pasture phenolics in addressing agroecological challenges. Future research should consider plant maturity's influence on metabolite composition, as well as anti-nutrient content and palatability of pastures. An untargeted LC-MS/MS approach should be explored to further investigate the phenolic profile of diverse swards.

Acknowledgement

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