The Effect of the Invasive Plant Species
*Lupinus polyphyllus* Lindl. on Energy Recovery
Parameters of Semi-Natural Grassland Biomass

Frank Hensgen * and Michael Wachendorf

Department of Grassland Science and Renewable Plant Resources, University of Kassel, Steinstraße 19, 37213 Witzenhausen, Germany; mwach@uni-kassel.de

* Correspondence: hensgen@uni-kassel.de; Tel.: +49-5542-98-1245

Academic Editor: Shelley Burgin

Received: 12 July 2016; Accepted: 28 September 2016; Published: 7 October 2016

**Abstract:** Biodiversity of semi-natural grasslands is increasingly endangered by successful invasive plant species such as the legume *Lupinus polyphyllus* Lindl. In order to contain the propagation of this plant species, early and regular harvesting needs to be applied. Therefore, a form of utilization for the harvested biomass has to be developed. One opportunity could be the use of the biomass as a feedstock for biogas and solid fuel production. This study investigates the effect of *L. polyphyllus* on the nutrient and mineral composition in a mixture series with semi-natural grassland biomass and examines the changes in nutrient and mineral content through hydrothermal conditioning and mechanical dewatering of silage. Untreated lupine-invaded biomass has higher N and Mg concentrations, but lower Cl, K and S concentrations compared to the semi-natural grassland biomass. The mineral concentrations in the biomass exceeded recommendations for combustion. However, with the proposed pre-treatment of hydrothermal conditioning and subsequent dewatering, both lupine-containing and lupine-free semi-natural grassland biomass could achieve adequate values for combustion, given that a state-of-the-art combustion technique is used, including measures to reduce emissions of NO\textsubscript{x} and particulate matter. Thus, solid fuel production through hydrothermal conditioning and mechanical separation may offer a practical solution for the containment of lupine or other invasive species in semi-natural grasslands and may constitute an important element for sustainable bioenergy production.

**Keywords:** bioenergy; semi-natural grassland; invasive plant

1. Introduction

Semi-natural grasslands are hotspots of biodiversity but are increasingly endangered by intensification and cessation of traditional management [1,2]. In addition, invasive plant species such as the legume *Lupinus polyphyllus* Lindl. [3] (hereafter referred to as lupine) have spread in European semi-natural grasslands and contributed to a decrease in biodiversity [4]. The lupine is capable of fixing nitrogen due to its symbiosis with rhizobial bacteria. In recent years, the species has invaded semi-natural lower mountain grasslands, as e.g., the UNESCO biosphere reserve “Hohe Rhön” in the German states of Hesse, Bavaria and Thuringia [5]. These habitats harbor semi-natural grassland communities adapted to low nutrient availability, such as mountain hay meadows and species rich Nardus grasslands (NATURA habitat types 6520 and 6320, respectively). In a traditional management regime with 1–2 cuts per year, removal of biomass, and either no use of fertilizers or only a low dose application, biodiversity can be maintained and the invasive plant species can be kept under control [6,7]. However, the harvest of biomass heavily infested with alien plant species such as lupine in lower mountain ranges is costly, and often no utilization alternative is available. In the case of
lupine, utilization of the biomass as forage for ruminants is hindered by toxic alkaloids [8]. In order to maintain biodiversity in semi-natural grasslands of lower mountain regions, it is necessary to find a use for the biomass, which is flexible in terms of cutting date and forage quality, and also insensitive to toxic compounds. One option could be the Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB [9]). The system produces a press fluid (PF) for anaerobic digestion and a press cake (PC) for combustion. The biomass is conserved as silage, followed by a hydrothermal conditioning step, and a subsequent separation into a PF rich in minerals (S, P, K, Mg, K and Cl) and a PC rich in fibers. The PF is used in anaerobic digestion for biogas production and the PC is used to produce a solid fuel in the form of briquettes or pellets. The IFBB system has been investigated using semi-natural grassland biomass and has proven useful in the production of a low mineral solid fuel by high mass flows of elements detrimental for combustion into the PF [10]. The resulting PF is an easily degradable input for anaerobic digestion [11]. Additionally, it has been shown that this energy conversion system can accommodate material from a wide range of cutting dates, and can therefore help to maintain biodiversity in semi-natural grasslands [12]. The IFBB thus combines nature conservation with a sustainable means of energy production. Since only biomass from nature conservation is used, which is not utilized in any other way, competition with feed or food production is avoided. However, there is no research available so far on the effect of lupine on the feasibility of the energy recovery system. Hence, within this study, we produced an artificial mixture series of lupine with semi-natural grassland biomass to investigate the effect of lupine on:

(i) The nutrient and mineral composition of the silage,
(ii) The mass flows of elements into the press fluid of the IFBB system,
(iii) The concentration of minerals in the press cake,
(iv) The energetic parameters for anaerobic digestion and combustion.

2. Materials and Methods

2.1. Site Characterisation and Harvest of Sample Biomass

The experimental site was located in the “Rhön” UNESCO biosphere reserve. Two biomass types were collected in June 2014: grassland biomass without lupine (L0) and pure lupine biomass (L100). Grassland biomass free of lupine was collected from the edge of a typical “Rhön” grassland area of the association “Geranio sylvatici–Trisetetum”. A 3 m by 100 m strip was cut with a finger bar mower with a stubble height of 5 cm. Approximately 200 kg fresh matter (FM) was harvested. The lupine plants were manually separated from the lupine infested sward and piled in heaps for further processing. Approximately 300 kg FM of lupine biomass was harvested.

2.2. Processing of the Biomass

2.2.1. Ensiling and Preparation of a Mixture Series

Both biomass types were left to wilt in the open air over night. Afterwards, they were chopped separately with a maize chopper and ensiled in three replicate 30 L polyethylene barrels for each raw material (L0 and L100). A series of mixtures of both biomass types was also ensiled with three replicates for each combination, including 25%, 50% and 75% lupine fresh weight and 75%, 50% and 25% grassland biomass, respectively, named L25, L50 and L75.

2.2.2. Hydrothermal Conditioning and Mechanical Separation

After ensiling for at least six weeks, samples were opened and thoroughly mixed, and the net weight was measured. In addition, 2 kg of each sample were taken for determination of the biogas potential of the silage. Another two subsamples were utilized for determination of dry matter and chemical analysis. Furthermore, 10–20 kg of each sample were mixed with 40 °C water in a ratio of 1:4 (silage:water) in a modified concrete mixer with a maximum volume of 200 L. The mash was kept
at a constant temperature of 40 °C with gas burners and stirred for 15 min. Subsequent mechanical dehydration of the silage was conducted with a screw press (type AV, Anhydro Ltd., Kassel, Germany). The conical screw had a pitch of 1:6 and a rotational speed of 6 rev·min⁻¹. The cylindrical screen encapsulating the screw had a perforation of 1.5 mm. Samples of the PC were taken for dry matter and chemical analysis.

Samples of the silage and the PF were stored at −18 °C prior to anaerobic digestion experiments. A subsample of the silage and the PC were immediately dried at 60 °C for 24 h for chemical analysis.

2.3. Chemical Analysis and Calculation of Mass Flows

The DM of the silage, PC and PF was determined by oven drying at 105 °C for 48 h. Total ash concentration was determined by combustion in a muffle furnace at 550 °C for 48 h. The dried samples (60 °C, 48 h) were analysed for C, H and N using an elemental analyser (Vario MAX CHN, Elementar Analysensysteme GmbH, Hanau, Germany). S, K, P, Cl, Mg and Ca were analysed using X-ray fluorescence analysis.

Mass flows of organic compounds from the silage into the PC and PF were calculated according to the formulae:

\[ MF_{X_{PC}} = \frac{M_{PC} \times X_{PC}}{M_{Sil} \times X_{Sil}} \]  

\[ MF_{X_{PF}} = 1 - MF_{X_{PC}} \]

with:

\( MF_{X_{PC}} \): Mass flow of a specific element \( X \) into the press cake,
\( M_{PC} \): Weight of the press cake in kg,
\( X_{PC} \): Concentration of a specific element \( X \) in the press cake in g·kg⁻¹,
\( M_{Sil} \): Weight of the silage in kg,
\( X_{Sil} \): Concentration of a specific element \( X \) in the silage in g·kg⁻¹,
\( MF_{X_{PF}} \): Mass flow of a specific element \( X \) into the press fluid.

2.4. Determination of Biogas Production from Silage and Press Fluid

Determination of the biogas production of the PF from the IFBB system and the silage samples was performed in batch experiments in accordance with the German Standard [13] (VDI 4630, 2004), with three replicates for PF and two replicates for silage. Fermentation of the substrates took place in gas-proof 20 L polyethylene containers. Mixing of the digester content was carried out for 15 min every 3 h. The experiments were performed at 37 °C, with a fluctuation of ±1 °C. Digested slurry from a biogas plant was used as an inoculant (8 kg FM). In addition, 4 kg FM of PF were used and the fermentation time was 14 days in the case of the PF. For the silage, 3.6 kg water and 0.4 kg silage were added and the fermentation time was 35 days. Measurement of gas fluxes started 24 h after incubation and was repeated once per workday. The total daily biogas volume was determined with a wet drum gas meter (TG1, Ritter Ltd., Bochum, Germany). The biogas composition (percentage of CH₄) was measured with an infrared spectrometer (GS Messtechnik, type GS IRM 100, Ratingen, Germany). Methane volumes were measured under laboratory room conditions, converted to standard conditions (273.15 K, 101.325 kPa) and expressed as normal liters (Lₙ). These methane volumes were referred to as methane yields when they were related to the amount of volatile solids (VS). It is documented that the content of dry matter in the silage is higher than the dry matter determined by oven drying because of volatile components lost during the drying procedure [14]. To correct the oven-measured DM content, analysis of volatile components was carried out by an external laboratory and the DM content was corrected according to the following formulae for silage (3) and press fluid (4) (all concentrations in g·kg⁻¹ FM):

\[ DM_{Sil,corr} = DM_{Sil} + (1.05 - 0.059 \times pH) \times OA + 0.08 \times LA + A \]  

(3)
with:

- $DM_{SIL_{corr}}$: DM of silage after correction,
- $DM_{Sil}$: DM of silage obtained by oven drying,
- $pH$: pH value of the silage,
- $OA$: sum of concentration of organic acids not including lactic acid,
- $LA$: concentration of lactic acid,
- $A$: Sum of concentration of alcohols,

\[
DM_{PF_{corr}} = DM_{PF} + (1.05 - 0.059 \times pH) \times OA + 0.08 \times LA + A, \tag{4}
\]

with:

- $DM_{PF_{corr}}$: DM of press fluid after correction,
- $DM_{PF}$: DM of press fluid obtained by oven drying,
- $pH$: pH value of the press fluid,
- $OA$: sum of concentration of organic acids not including lactic acid,
- $LA$: concentration of lactic acid,
- $A$: Sum of concentration of alcohols.

2.5. Calculation of Higher Heating Value and Theoretical Gross Energy Yields

Gross energy yields for IFBB and anaerobic digestion of silage were calculated. The basis for the substrate-related calculation was one kg of silage DM, excluding losses through harvest and conversion technology. For the IFBB system, energy contained in the silage and PC was calculated as the higher heating value (HHV, MJ·kg$^{-1}$ DM). The calculation was based on the concentrations of C, H, and N (g·kg$^{-1}$ DM) using the following equation for bio-fuels by Friedl et al. [15]:

\[
HHV = 3.55C^2 - 232 \times C - 2230 \times H + 51.2 \times C \times H + 131 \times N + 20600. \tag{5}
\]

The lower heating value (LHV, MJ·kg$^{-1}$ DM) was calculated from the higher heating value (HHV) by taking the enthalpy of water vaporization into account:

\[
LHV = HHV - \left(8.937 \times \frac{H}{100}\right) \times 2.2. \tag{6}
\]

As a second energy carrier in the IFBB system, the energy contained in the PF was calculated. The measured CH$_4$ yield of the PF was multiplied with the heating value of methane (36.4 MJ·m$^3$ CH$_4$). The total IFBB gross energy yield was calculated as the gross energy yield of PC plus PF. The gross energy yield for the silage in direct anaerobic digestion was calculated by multiplying the measured methane yields with the heating value of methane.

2.6. Statistical Analysis

Statistical analyses were done using the Software R (Version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria) [16]. Analysis of variance (ANOVA) was performed to test the effect of lupine on the chemical composition (DM, $XA$, N, S, K, Ca, Mg, Cl, P) of the raw material and PC, as well as the effect of lupine on mass flows within the IFBB system, the effect of lupine on silage and PF methane yield, and the effect of lupine on silage and the PC higher heating value. Assumptions of the ANOVA were tested, and the only violation was the assumption of normality for the higher heating value of the press cake. We decided to use the ANOVA for this parameter anyway, as several authors have pointed out that ANOVA is quite robust to violations of this assumption [17]. The Tukey honestly significant difference (HSD) test was carried out as a post hoc test for all ANOVAs.
3. Results

3.1. Chemical Composition of the Silage and Press Cake

There was a clear effect of lupine content on dry matter and mineral concentration in the silages (Figure 1). Silage made of pure lupine showed significantly lower DM concentrations compared to silage without lupine (15.67% vs. 30.58% for L100 and L0, respectively). Logically, mixed silages had intermediate DM values (19.19%, 23.50%, 26.07% for L75, L50 and L25, respectively). The same trend was observed for concentrations of S, K, Cl (Figure 1) and P (0.16%, 0.18%, 0.21%, 0.22%, 0.23% DM for L100, L75, L50, L25, L0). The opposite trend of increasing concentrations with increasing lupine content was found for total ash, N and Ca (Figure 1), and Mg (0.56%, 0.42%, 0.37%, 0.32%, 0.28% DM for L100, L75, L50, L25, L0). All observed differences were highly significant. Thus, it can be stated that the invasion of grasslands by lupine leads to increasing concentrations of total ash, N, Ca and Mg in the silage, while simultaneously lowering concentrations of S, K, Cl and P compared to silage from non-invaded semi-natural grassland.

Figure 1. Arithmetic means of concentration of ash (XA), Nitrogen (N), Sulphur (S), Calcium (Ca), Potassium (K) and Chlorine (Cl) given in % dry matter (DM) in silage and press cake from grassland biomass with lupine content between 0 (L0) and 100% fresh matter (FM) (L100). Error bars depict the standard error of the means.
The IFBB treatment led to lower concentrations of total ash, minerals and N in the PC compared to the silage (Figure 1), whereas only smaller differences could be found in DM, total ash and sulphur content (Table 1). The concentrations of N, K, Mg, Ca, Cl and P remained statistically different between the different raw materials (L0, L100). The PC stemming from biomass containing lupine showed significantly higher concentrations of N, Mg and Ca and significantly lower concentrations of K, Cl, and P compared to semi-natural grassland biomass PC without lupine. In the case of K, Cl and P, even the addition of 25% lupine changed the concentration significantly, whereas in the case of N, Mg and Ca, a significant difference only existed for the treatments with 50% lupine or more.

Table 1. Results of analysis of variance and post-hoc test for the effect of lupine content on mineral concentration in the silage and press cake. Different letters indicate significant differences between groups, n.s. = not significant.

|          | Silage       | Press Cake  |
|----------|--------------|-------------|
|          | p L0 L25 L50 L75 L100 | p          |
| DM       | <0.001       | a b c d e   | 0.78       | n.s. | n.s. | n.s. | n.s. | n.s. |
| Ash      | <0.001       | b b b b a   | 0.34       | n.s. | n.s. | n.s. | n.s. |
| N        | <0.001       | e d c b a   | <0.001     | c    | c    | b    | a    |      |
| S        | <0.001       | a ab b cb c | 0.08       | n.s. | n.s. | n.s. | n.s. |      |
| K        | <0.001       | a b c d e   | <0.001     | a    | b    | c    | d    |
| Mg       | <0.001       | e d c b a   | <0.001     | cd   | d    | c    | b    |
| Ca       | <0.001       | d c b a     | <0.001     | d    | d    | c    | b    |
| Cl       | <0.001       | a b c d e   | <0.001     | a    | b    | b    | c    |
| P        | <0.001       | a b c d    e | <0.001     | a    | b    | b    |

3.2. Mass Flows into Press Liquid

Table 2 shows the results for the mass flows of dry matter, ash, nitrogen and minerals into the PF within the IFBB system. A high mass flow of ash, N, S, K, Mg, and Cl is desirable to achieve a PC with reduced concentrations of these elements, and, therefore, a lower risk of emissions and ash slagging, and increased heating value. A reduction in element concentration during IFBB processing only applies if the mass flow of a specific element into the PF is higher than that of the DM. This was valid for all investigated chemical components. Mass flows were lower for the 100% grassland biomass than for the 100% lupine biomass, and the values of the mixtures L25, L50 and L75 were in between those of the pure raw materials. However, only the differences for N, K, Mg, Ca and P were of statistical significance. The mixtures and L100 were often not statistically different from one another, whereas the addition of only 25% lupine to the grassland biomass already resulted in significantly increased mass flows for K, Mg and Ca (Table 2).

Table 2. Mass flow of DM, ash, N, S, K, Mg, Ca, Cl and P into the press fluid (arithmetic mean values in% ± standard error of means) for silages from grassland biomass with lupine content between 0 (L0) and 100% FM (L100). Upper case letters indicate significant differences between groups.

|          | L0     | L25     | L50     | L75     | L100    |
|----------|--------|---------|---------|---------|---------|
| DM       | 15.6 ± 1.5 | 25.9 ± 5.5 | 25.4 ± 4.5 | 25.4 ± 4.3 | 31.5 ± 3.2 |
| Ash      | 48.0 ± 1.2 | 56.6 ± 3.4 | 56.5 ± 2.3 | 59.8 ± 1.9 | 61.2 ± 4.9 |
| N        | 39.5 ± 1.2 b | 48.4 ± 4.2 ab | 46.2 ± 4.4 ab | 51.1 ± 2.0 ab | 55.5 ± 2.1 a |
| S        | 47.8 ± 2.2 | 55.9 ± 4.3 | 49.4 ± 5.9 | 45.2 ± 2.7 | 52.7 ± 4.9 |
| K        | 85.9 ± 0.3 c | 90.0 ± 0.8 b | 90.5 ± 0.5 b | 91.6 ± 0.3 ab | 93.3 ± 0.4 a |
| Mg       | 63.6 ± 1.1 c | 73.8 ± 1.3 b | 75.0 ± 1.7 b | 76.9 ± 0.9 ab | 81.4 ± 1.2 a |
| Ca       | 28.2 ± 3.5 c | 45.7 ± 2.2 b | 50.6 ± 3.8 ab | 53.3 ± 2.2 ab | 63.5 ± 2.9 a |
| Cl       | 93.3 ± 0.1 | 95.3 ± 0.6 | 94.5 ± 0.3 | 95.4 ± 0.2 | 94.9 ± 1.0 |
| P        | 78.9 ± 0.4 b | 83.7 ± 1.6 ab | 82.2 ± 1.3 ab | 83.1 ± 0.7 ab | 86.2 ± 1.0 a |
3.3. Energetic Parameters

3.3.1. Anaerobic Digestion

Anaerobic digestion tests with the silages showed mean methane yields between 251 and 270 L\textsubscript{N}·kg\textsuperscript{-1} VS (Figure 2), with no difference between the levels of lupine content (Table 3). Correspondingly, the degree of degradation of VS did not differ between the groups of the mixture series (Table 3), with mean values of 59.0%, 57.2%, 60.1%, 60.4% and 62.0% for L0, L25, L50, L75 and L100, respectively. Mean methane yields of the press fluid were between 334 and 434 L\textsubscript{N}·kg\textsuperscript{-1} VS (Figure 2), and the L50 mixture showed lower values than both raw materials and the other mixtures (Table 3). The mean values for the degree of degradation of VS were 98.4%, 92.4%, 80.3%, 87.9% and 90.9% for L0, L25, L50, L75 and L100, respectively.

Figure 2. Methane yields of silage and press fluid (a), and higher heating values of silage and press cake (b) from grassland biomass with lupine content between 0 (L0) and 100% FM (L100). Error bars depict the standard error of the means.

Table 3. Results of analysis of variance and post-hoc test for the effect of lupine content on methane yield and degree of degradation of silage and press fluid, and the effect of lupine content on the higher heating value of silage and press cake. Different letters indicate significant differences between groups, n.s. = not significant.

|                  | Silage        | Press Fluid  | Press Cake   |
|------------------|---------------|--------------|--------------|
|                  | L0 L25 L50 L75 L100 | L0 L25 L50 L75 L100 | <0.001 b b a a a 0.009 b ab ab a a |
| p                |               |              |              |
| CH4 yield        | 0.97 n.s. n.s. n.s. n.s. 0.004 a a b ab a |              |              |
| Degree of degradation | 0.85 n.s. n.s. n.s. n.s. n.s. 0.006 a a b ab a |              |              |

3.3.2. Heating Values

The higher heating values of the silage were between 18.6 and 19.0 MJ·kg\textsuperscript{-1} DM, with the lowest value for L0 and the highest value for L75, caused by the higher N concentration of lupine biomass (Figure 2). The differences between L0 and L25 versus L50, L75 and L100 were statistically significant (Table 3). The press cake showed increased higher heating values in comparison to the silage (19.0 to 19.2 MJ·kg\textsuperscript{-1} DM) and a significant difference between L0 versus L75 and L100 could be observed, with the higher values for PCs from biomass with high lupine content. The corresponding lower heating values ranged from 17.4 (L0) to 17.8 (L75) MJ·kg\textsuperscript{-1} DM for the silages and 17.8 (L0) to 18.0 (L100) MJ·kg\textsuperscript{-1} DM for the PC.
3.3.3. Calculated Gross Energy Yields

Substrate-related gross energy yields for direct anaerobic digestion of the silage ranged between 8.36 and 8.99 MJ·kg\(^{-1}\) silage DM (Figure 3), with no effect of lupine content (Table 4). However, for the IFBB system, an effect of the lupine was shown. The gross energy yield of anaerobic digestion of the PF showed lower substrate-related gross energy yields, as only 16% to 32% of the DM was washed into the PF (Table 2). Energy yields were between 2.56 (L0) and 4.59 (L100) MJ·kg\(^{-1}\) silage DM, with significant differences between the groups, i.e., lupine-rich materials (L100, L75) achieved higher values than lupine-poor materials (L0, L25, L50). The opposite effect was found for the gross energy yield of the PC, which decreased from 15.10 (L100) to 12.48 (L0) MJ·kg\(^{-1}\) silage DM. This led to cumulative gross energy yields of PC and PF between 16.53 (L50) and 17.66 (L0) MJ·kg\(^{-1}\) silage DM, with lower values for L50 and L75, higher values for L100 and L25, and the highest value for L0.

![Figure 3](image-url)  
*Figure 3.* Calculated gross energy yield (MJ·kg\(^{-1}\) silage DM) from anaerobic digestion of silage as well as press fluid and press cake originating from grassland biomass with lupine content between 0 (L0) and 100% FM (L100). Error bars depict the standard error of the means.

|          | AD  | L0   | L25  | L50  | L75  | L100 |
|----------|-----|------|------|------|------|------|
| p        | 0.971 | n.s. | n.s. | n.s. | n.s. | n.s. |
| IFBB PF  | <0.001 | c    | c    | c    | c    | a    |
| IFBB PC  | <0.001 | a    | ab   | b    | bc   | c    |
| IFBB PC + PF | 0.005 | a    | ab   | c    | bc   | abc  |

4. Discussion

4.1. Chemical Composition of Silage and Press Cake

The lupine biomass and the mixtures showed higher concentrations of total ash, Ca, Mg, and N and lower concentrations of S, K, Cl, and P than the semi-natural grassland silages. Compared to other research results from semi-natural grassland biomass [9,10,18,19], the mineral concentration values for the L0 biomass are within the expected range for biomass harvested in June; however, the values for the L100 samples for Ca, Mg and N were considerably higher. This corresponds with previous findings, which likewise found higher mineral content, particularly for Ca, Mg and N [20,21], in legumes and other dicotyledonous plants compared to grasses. However, the observed values for...
mineral composition, especially for N, K, Mg, S and Cl, led to the conclusion that a direct combustion of the material, be it lupine, semi-natural grassland biomass, or any mixture of the two, is not advisable. Specifically, the concentration of S, N and Cl (0.1%, 0.6%, 0.1% DM, respectively) are above the guiding values proposed by Obernberger et al. [22] for an unproblematic combustion process. The IFBB method reduced the concentration of S and Cl below the threshold values for all samples, but the N concentrations were still higher than recommended. Thus, the IFBB fuel is feasible for combustion, but technical measures are necessary to reduce NO\(_x\) emissions. Air staging has proved to be an efficient means of reducing NO\(_x\) emissions drastically [23,24].

4.2. Mass Flows into Press Liquid

We found a significant influence of lupine on mass flows, with lupine content increasing the mass flow into the press liquid for N, K, Mg, Ca and P. This is in line with our expectations, considering the significantly lower DM concentration in lupine biomass compared to grassland material. Richter et al. [18] have shown that DM content in the silage has a negative impact on mass flows of minerals into PF within the IFBB system, thus resulting in higher mass flows for silages with low DM concentration and vice versa. Low DM content in the silage may lead to a soaked condition of the cell walls, which results in a higher degree of maceration during the press process. Cell water may further contribute to the rupture of cell walls during compression, resulting in an enhanced discharge of intercellular compounds [18].

4.3. Energetic Parameters

4.3.1. Anaerobic Digestion

The methane yields of the silage samples, between 251 and 271 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS, are within the range for silages from extensively managed grassland areas observed in earlier studies. Wachendorf et al. [9] observed methane yields with a mean value of 218 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS for five semi-natural grassland areas, and Piepenschneider et al. [25] measured values of 222 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS for urban road-side cut material from the city of Kassel, Germany. While these values were slightly lower than the values reported in this study, higher methane yields (285–388 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS) have also been reported by Richter et al. [26] for samples from an alluvial meadow with successive harvesting dates, with the tendency of decreasing methane yields with progressing sward maturity.

There was no effect of lupine on silage methane yields and the degree of degradation; therefore, the higher nitrogen concentration in the lupine biomass did not lead to higher methane yields. Whether this is due to a lower concentration of other easily degradable components in lupine biomass or due to an inhibitory effect of the alkaloids present in the lupine biomass needs further investigation. Previous studies reported that alkaloids in lupine, like lupanine and lupinine, may have a negative, bactericide-like effect on microbes [27].

The methane yields for the PF were in accordance with findings in earlier investigations. Hensgen et al. [11], for example, found mean PF methane yields of 353 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS from German semi-natural grassland areas, whereas Wachendorf et al. [9] found a mean PF methane yield of 426 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS from five semi-natural grassland sites from southern Germany. Bühle et al. conducted a comprehensive study on the digestion of PF using different types of digesters, and found methane yields between 390 and 506 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS for PF resulting from maize silage as an input material, and the degree of degradation of the VS amounted to more than 90% [28]. The IFBB PF from private and communal urban green cuttings from the German city of Baden-Baden also showed a methane yield of 350 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS and a degree of degradation of 81% [29]. Piepenschneider et al. investigated the use of autumn leaves from five tree species for the IFBB system, and found extremely low PF methane yields (172 L\(_{\text{N}}\)·CH\(_4\)·kg\(^{-1}\) VS) and degrees of degradation (43%) [30].

From this, it can be concluded that a high digestibility and methane yield of the raw material leads to an increased digestibility and methane yield of the PF. As the raw materials and mixtures
did not differ in digestibility or methane yield, we did not expect to find a large variation in methane yield of the PF between the raw materials and the mixtures. However, significantly lower methane yields and degrees of degradation were found for the L50 mixture compared to the other treatments. This could be due to errors in measurement or a consequence of processes within the screw press in the IFBB method that we are not aware of so far, which could explored by further research.

4.3.2. Heating Value

Higher heating values found in this study (18.6 to 19.0 MJ·kg$^{-1}$ DM for silage and 19.0 to 19.2 MJ·kg$^{-1}$ DM for PC) were comparable with values found in earlier studies on energetic conversion of grassland silages [26,31]. The HHVs found by Richter et al. [31] were a bit lower, with 17.8 MJ·kg$^{-1}$ DM for silages and 18.4 MJ·kg$^{-1}$ DM for PC. While these values were calculated using the chemical composition of biomass, Khalsa et al. [32] conducted measurements in a bomb calorimeter with leached biomass comparable to the IFBB PC described here. They found LHV values of 18.4, 18.9 and 18.2 MJ·kg$^{-1}$ DM for leached grass, foliage and a 50%:50% mixture, which is only marginally higher than the calculated LHVs for L0, L25, L50, L75 and L100 (17.8, 17.9, 18.0, 18.0, and 18.0 MJ·kg$^{-1}$ DM, respectively).

The PCs always showed an increased higher heating value in comparison to the silages, as a result of lower ash concentration in the PC due to leaching of mineral elements during the IFBB process. In comparison to other biomass fuels (Wheat straw: 18.5 MJ·kg$^{-1}$ DM, Miscanthus: 19.1 MJ·kg$^{-1}$ DM, short rotation poplar: 19.8 MJ·kg$^{-1}$ DM, spruce wood: 20.2 MJ·kg$^{-1}$ DM [33]), it can be concluded that the HHVs for silage from semi-natural grassland are between wheat straw and miscanthus, whereas the PC heating values are between miscanthus and wood from short rotation coppices. The lupine content had a positive effect on the heating values of the silage and press cake due to the higher N concentration.

4.3.3. Calculated Gross Energy Yield

Gross energy yields were comparable to earlier results published by Hensgen et al. [11] for a range of biomass from Estonian, Welsh and German semi-natural grasslands, although these values were calculated on the basis of DM biomass instead of DM silage. The values for anaerobic digestion of silage (8.36–8.99 MJ·kg$^{-1}$ silage DM) are in between the results from Hensgen et al. (6.48–9.00 MJ·kg$^{-1}$ DM biomass) [11]. Energy yields for press fluids (2.56–4.59 MJ·kg$^{-1}$ silage DM) in the present study were higher than those found by Hensgen et al. [11] (1.04–2.48 MJ·kg$^{-1}$ DM biomass), mainly caused by higher mass flows of DM into PF, but also through slightly higher methane yields of the PF. The higher mass flow of DM into PF also explains the somewhat lower gross energy yields of the PC in the actual study (12.48–15.10 MJ·kg$^{-1}$ silage DM) compared to Hensgen et al. (14.51–18.79 MJ·kg$^{-1}$ DM biomass) [11]. However, cumulative gross energy yield of the IFBB system is within the same range in both studies. These values represent gross energy, meaning that the input side of the energy balance was not measured and taken into account. However, by applying data for the conservation losses and conversion system, as well as data on specific energy input in direct anaerobic digestion and IFBB, as reported by Bühle et al. [34], net energy yields can be calculated from gross energy yields. Bühle et al. [34] showed that the net conversion efficiency of the IFBB system was between 44.7% and 52.9%, meaning that about half of the gross energy contained in the biomass is converted into heat by the IFBB system. Considering the effect of the lupine, we found that increasing lupine contribution in the biomass led to higher energy yields from the press fluid and lower energy yields from the PC. For the cumulative energy yield, it can be stated that, although the differences are statistically significant, they are in such a minor range that they will not play a role in large-scale energy conversion systems. Thus, both the lupine and lupine-free semi-natural grassland biomass yield a significant amount of energy if the IFBB system is applied.
5. Conclusions

In conclusion, it can be stated that:

(i) There was an effect of lupine on nutrient and mineral composition of the silage, with higher concentrations of total ash, N, Ca and Mg, but also significantly lower DM, S, K, Cl and P concentrations compared to semi-natural grassland silage.

(ii) There was an effect of lupine on the mass flow of elements into press fluid after washing and mechanical dehydration, with higher mass flows of N, K, Mg, Ca and P for lupine material.

(iii) There was an effect of lupine on the concentration of minerals in the press cake. The press cakes from biomass containing lupine showed significantly higher concentrations of N, Mg and Ca and significantly lower concentrations of K, Cl, and P compared to semi-natural grassland biomass without lupine.

(iv) There was no effect of lupine on methane yields and degree of degradation of silages. However, methane yields of press fluids from the 50% mixture of lupine and grassland biomass showed significantly lower methane yields and degrees of degradation in anaerobic digestion. Within the IFBB system, increased lupine content led to higher gross energy yields from PF, but lower gross energy yields from PC. In total, the gross energy yields were best for the lupine-free semi-natural grassland biomass and worst for the 50% mixture, but differences were only marginal.

Thus, solid fuel production through hydrothermal conditioning and mechanical separation may offer a solution for the containment of lupine or other invasive species in semi-natural grasslands. Contrary to animal feeding or anaerobic digestion, where lupine seeds may remain vigorous and invade agricultural land when broadcasted with residues from those processes (like manures and digestates), seeds will definitely not survive the combustion process of press cakes. Hence, IFBB may support several valuable ecosystem services of grassland habitats, i.e., (i) climate change mitigation (through the generation of renewable energy); (ii) biodiversity (though the control of an invasive plant and flexible harvest schedules, allowing the maintenance of flowering species as a nutritional basis for threatened pollinators); and (iii) non-material benefits people obtain from intact grassland ecosystems (e.g., reflection, recreation, and aesthetic experience). In summary, the suggested management of lupine-invaded grasslands with the IFBB process may constitute an important element for sustainable bioenergy production.

Acknowledgments: The authors received funding from the “Deutsche Bundesstiftung Umwelt” (German Federal Environmental Foundation).

Author Contributions: Frank Hensgen and Michael Wachendorf conceived and designed the experiments; Frank Hensgen performed the experiments; Frank Hensgen and Michael Wachendorf analyzed the data, Frank Hensgen is the main author of the paper, Michael Wachendorf co-authored the paper.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

- A: Alcohol
- HHV: Higher heating value
- IFBB: Integrated generation of solid fuel and biogas from biomass
- LA: Lactic acid
- LHV: Lower heating value
- L0: Biomass sample containing 0% lupine and 100% semi-natural grassland biomass
- L25: Biomass sample containing 25% lupine and 75% semi-natural grassland biomass
- L50: Biomass sample containing 50% lupine and 50% semi-natural grassland biomass
- L75: Biomass sample containing 75% lupine and 25% semi-natural grassland biomass
- L100: Biomass sample containing 100% lupine and 0% semi-natural grassland biomass
- OA: Organic acids
- PF: Press fluid
- PC: Press cake
- VS: Volatile solids
- XA: Ash
### Appendix A

#### Table A1. Mean values of elements in silage and PC samples with lupine content between 0 L0 and 100% FM (L100).

|       | DM  | Ash | N   | S   | K   | Mg  | Ca  | Cl  | P   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|       | % FM| % DM| % DM| % DM| % DM| % DM| % DM| % DM| % DM|
| L100  |     |     |     |     |     |     |     |     |     |
| Silage| 15.82 | 8.98 | 2.43 | 0.10 | 0.95 | 0.58 | 2.14 | 0.12 | 0.17 |
| Silage| 15.61 | 9.31 | 2.36 | 0.10 | 0.91 | 0.56 | 1.95 | 0.11 | 0.16 |
| Silage| 15.58 | 8.92 | 2.38 | 0.10 | 0.92 | 0.54 | 1.85 | 0.11 | 0.16 |
| L75   |     |     |     |     |     |     |     |     |     |
| Silage| 19.71 | 8.44 | 1.93 | 0.11 | 1.21 | 0.41 | 1.33 | 0.40 | 0.18 |
| Silage| 19.95 | 8.35 | 1.98 | 0.11 | 1.22 | 0.42 | 1.37 | 0.39 | 0.18 |
| Silage| 17.92 | 8.53 | 2.02 | 0.11 | 1.25 | 0.45 | 1.40 | 0.40 | 0.19 |
| L50   |     |     |     |     |     |     |     |     |     |
| Silage| 23.22 | 8.12 | 1.74 | 0.12 | 1.54 | 0.37 | 1.10 | 0.61 | 0.21 |
| Silage| 23.41 | 8.17 | 1.72 | 0.11 | 1.47 | 0.36 | 1.08 | 0.59 | 0.20 |
| Silage| 23.87 | 8.11 | 1.72 | 0.12 | 1.51 | 0.37 | 1.09 | 0.62 | 0.21 |
| L25   |     |     |     |     |     |     |     |     |     |
| Silage| 26.90 | 8.17 | 1.57 | 0.13 | 1.70 | 0.31 | 0.80 | 0.77 | 0.22 |
| Silage| 25.95 | 8.23 | 1.58 | 0.12 | 1.74 | 0.33 | 0.84 | 0.77 | 0.22 |
| Silage| 25.37 | 8.06 | 1.57 | 0.12 | 1.70 | 0.33 | 0.88 | 0.74 | 0.22 |
| L0    |     |     |     |     |     |     |     |     |     |
| Silage| 30.50 | 8.23 | 1.45 | 0.14 | 1.88 | 0.28 | 0.66 | 0.90 | 0.24 |
| Silage| 31.31 | 8.25 | 1.45 | 0.14 | 1.89 | 0.29 | 0.69 | 0.91 | 0.23 |
| Silage| 29.92 | 8.44 | 1.47 | 0.12 | 1.87 | 0.28 | 0.62 | 0.90 | 0.23 |
| L100  |     |     |     |     |     |     |     |     |     |
| PC    | 44.47 | 6.07 | 1.50 | 0.07 | 0.09 | 0.15 | 1.07 | 0.01 | 0.03 |
| PC    | 43.56 | 4.68 | 1.57 | 0.06 | 0.09 | 0.15 | 1.01 | 0.01 | 0.03 |
| PC    | 45.38 | 4.59 | 1.59 | 0.07 | 0.09 | 0.15 | 1.07 | 0.01 | 0.03 |
| L75   |     |     |     |     |     |     |     |     |     |
| PC    | 44.19 | 4.57 | 1.32 | 0.08 | 0.14 | 0.13 | 0.83 | 0.03 | 0.04 |
| PC    | 44.92 | 4.59 | 1.29 | 0.09 | 0.14 | 0.14 | 0.89 | 0.02 | 0.04 |
| PC    | 45.85 | 4.49 | 1.29 | 0.07 | 0.14 | 0.13 | 0.85 | 0.02 | 0.04 |
| L50   |     |     |     |     |     |     |     |     |     |
| PC    | 42.20 | 4.73 | 1.19 | 0.07 | 0.20 | 0.12 | 0.69 | 0.05 | 0.05 |
| PC    | 48.60 | 4.71 | 1.28 | 0.08 | 0.19 | 0.12 | 0.74 | 0.04 | 0.05 |
| PC    | 43.54 | 4.81 | 1.26 | 0.08 | 0.20 | 0.12 | 0.73 | 0.04 | 0.05 |
| L25   |     |     |     |     |     |     |     |     |     |
| PC    | 48.17 | 4.69 | 1.07 | 0.07 | 0.22 | 0.11 | 0.62 | 0.04 | 0.05 |
| PC    | 44.31 | 4.80 | 1.11 | 0.07 | 0.24 | 0.11 | 0.58 | 0.05 | 0.05 |
| PC    | 46.41 | 4.81 | 1.10 | 0.08 | 0.23 | 0.12 | 0.64 | 0.05 | 0.05 |
| L0    |     |     |     |     |     |     |     |     |     |
| PC    | 45.31 | 5.26 | 1.07 | 0.08 | 0.31 | 0.12 | 0.56 | 0.07 | 0.06 |
| PC    | 44.37 | 5.12 | 1.06 | 0.08 | 0.32 | 0.12 | 0.56 | 0.07 | 0.06 |
| PC    | 45.39 | 4.97 | 1.01 | 0.08 | 0.32 | 0.12 | 0.55 | 0.07 | 0.06 |

### References

1. Poschlod, P.; Bakker, J.P.; Kahmen, S. Changing land use and its impact on biodiversity. Basic Appl. Ecol. 2005, 6, 93–98. [CrossRef]
2. Ostermann, O.P. The need for management of nature conservation sites designated under Natura 2000. J. Appl. Ecol. 1998, 35, 968–973. [CrossRef]
3. Lambdon, P.W.; Pysek, P.; Basnou, C.; Hejda, M.; Arianatsou, M.; Essl, F.; Jarosik, V.; Pergl, J.; Winter, M.; Anastasiu, P.; et al. Alien flora of Europe: Species diversity temporal trends, geographical patterns and research needs. Preslia 2008, 80, 101–149.
4. Lapin, K.; Bernhardt, K.G.; Lichtenwöhrer, P.; Roithmayr, S. Welchen Einfluss haben invasive Pflanzenarten auf die Phytodiversität von renaturierten Flusslandschaften? Gesunde Pflanz. 2015, 67, 75–82. (In German) [CrossRef]
5. Otte, A.; Maul, P. Verbreitungsschwerpunkte und strukturelle Einmischung der Stauden-Lupine (Lupinus polyphyllus Lindl.) in Bergwiesen der Rhön. Tuexenia 2005, 25, 151–182. (In German)
6. Jaquemyn, H.; Brys, R.; Hermy, M. Short-term effects of different management regimes on the response of calcareous grassland vegetation to increased nitrogen. 
   *Biol. Conserv.* 2003, 111, 137–147. [CrossRef]

7. Zechmeister, H.G.; Schmitzberger, I.; Steurer, B.; Peterseil, J.; Wrbka, T. The influence of land-use practices and economics on plant species richness in meadows. 
   *Biol. Conserv.* 2003, 114, 165–177. [CrossRef]

8. Veen, G.; Schmidt, C.; Witte, L.; Wray, V.; Czygan, F.C. Lupin alkaloids from *Lupinus polyphyllus*. 
   *Phytochemistry* 1992, 31, 4343–4345. [CrossRef]

9. Schmider, E.; Ziegler, M.; Danay, E.; Beyer, L.; Bühner, M. Is it really robust? Reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. 
   *Methodology* 2010, 6, 147–151. [CrossRef] [PubMed]

10. Obernberger, I.; Brunner, T.; Bärnthaler, G. Chemical properties of solid biofuels—Significance and impact. 
    *Biomass Bioenergy* 2006, 30, 973–982. [CrossRef]

11. Ruano-Ramos, A.; García-Ciudad, A.; García-Criado, B. Near infrared spectroscopy prediction of mineral content in botanical fractions from semi-arid grasslands. 
    *Anim. Feed Sci. Technol.* 1999, 77, 331–343. [CrossRef]

12. Pieperschneider, M.; Bühle, L.; Hensgen, F.; Wachendorf, M. Energy recovery from grass of urban roadside verges by anaerobic digestion and combustion after pre-processing. 
    *Biomass Bioenergy* 2016, 85, 278–287. [CrossRef]
26. Richter, F.; Fricke, T.; Wachendorf, M. Influence of sward maturity and pre-conditioning temperature on the energy production from grass slilage through integrated generation of solid fuel and biogas from biomass (IFBB): 2. Properties of energy carriers and energy yield. Bioresour. Technol. 2011, 102, 4866–4875. [CrossRef] [PubMed]

27. De la Vega, R.; Gutierrez, M.P.; Sanz, C.; Calvo, R.; Robredo, L.M.; de la Cuadra, C.; Muzquiz, M. Bactericide-like effect of Lupinus alkaloids. Ind. Crops Prod. 1996, 5, 141–148. [CrossRef]

28. Bühle, L.; Reulein, J.; Stülpnagel, R.; Zerr, W.; Wachendorf, M. Methane yields and digestion dynamics of press fluids from mechanically dehydrated maize silages using different types of digesters. Bioenergy Res. 2012, 5, 294–305. [CrossRef]

29. Hensgen, F.; Richter, F.; Wachendorf, M. Integrated generation of solid fuel and biogas from green cut material from landscape conservation and private households. Bioresour. Technol. 2011, 102, 10441–10450. [CrossRef] [PubMed]

30. Piepenschneider, M.; Nurmatov, N.; Bühle, L.; Hensgen, F.; Wachendorf, M. Chemical properties and ash slagging characteristics of solid fuels from urban leaf litter. Waste Biomass Valor. 2016, 7, 625–633. [CrossRef]

31. Richter, F.; Fricke, T.; Wachendorf, M. Utilization of semi-natural grassland through integrated generation of solid fuel and biogas from biomass. III. Effects of hydrothermal conditioning and mechanical dehydration on solid fuel properties and on energy and greenhouse gas balances. Grass Forage Sci. 2010, 65, 185–199. [CrossRef]

32. Khalsa, J.H.A.; Döhling, F.; Berger, F. Foliage and grass as fuel pellets—small scale combustion of washed and mechanically leached biomass. Energies 2016, 9, 361. [CrossRef]

33. Hartmann, H. Brennstoffzusammensetzung und -eigenschaften (Composition and characteristics of fuels). In Energie aus Biomasse: Grundlagen, Techniken und Verfahren, 2nd ed.; Kaltschmitt, M., Hartmann, H., Eds.; Springer: Berlin, Germany, 2009. (In German)

34. Bühle, L.; Hensgen, F.; Donnison, I.; Heinsoo, K.; Wachendorf, M. Life cycle assessment of the integrated generation of solid fuel and biogas from biomass (IFBB) in comparison to different energy recovery, animal-based and non-refining management systems. Bioresour. Technol. 2012, 111, 230–239. [CrossRef] [PubMed]

© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).