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1 **Effects of green manure herbage management and its digestate from biogas**
2 **production on barley yield, N recovery, soil structure and earthworm**
3 **populations**

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Abstract

In repeatedly mown and mulched green manure leys, the mulched herbage contains substantial amounts of nitrogen (N), which may only slightly contribute to the following crops' nutrient demand. The objective of the present work was to evaluate the effect of alternative strategies for green manure management on the yield and N recovery of a subsequent spring barley crop, and their short term effects on soil structure and earthworm populations. A field trial was run from 2008 to 2011 at four sites with contrasting soils under cold climate conditions. We compared several options for on-site herbage management and the application of anaerobically digested green manure herbage. Depending on the site, removal of green manure herbage reduced the barley grain yield by 0% to 33% compared to leaving it on-site. Applying digestate, containing 45% of the N in harvested herbage, as fertilizer for barley gave the same yields as when all herbage was mulched the preceding season. Overall, the apparent N recovery was enhanced from 7% when all herbage was mulched, to 16% when returned as digestate. A positive effect on earthworm density and biomass was seen after one season of retaining mulch material, rather than removing it. Digestate did not affect the earthworm population, but contributed to higher soil aggregate stability. In conclusion, for spring barley production after green manure ley, the digestate strategy increased N recovery and reduced the risk of N losses. The yield of the succeeding barley crop yield was reduced when N in herbage was not returned as mulch or digestate.

Key words

Organic stockless farming, ley, mulch, biogas residue, cereals, N use efficiency

59 **1. Introduction**

60 Green manure leys are commonly used in organic cereal crop rotations to maintain soil fertility on stockless
61 farms. Such full season grass-clover leys may increase yields through improved nitrogen (N) supply and through
62 non-nutritional benefits such as improved soil structure, suppression of diseases and weeds, more earthworms
63 and increased mycorrhizal activity (Cherr et al., 2006; Janzen and Schaalje, 1992; Riley et al., 2008). The green
64 manure leys are generally grown as set-aside; managed by leaving the chopped herbage as mulch after frequent
65 mowing during the growing season (Cormack et al., 2003; Stopes et al., 1996). The mowing is done as a means
66 to control weeds and to keep the clover in a vegetative state and thus sustain high N₂ fixating activity and low
67 C/N ratio (Dahlin and Stenberg, 2010).

68 Due to the accumulation of easily degradable N in green manure crops, current practice with repeatedly
69 mowing and mulching means that substantial amounts of N in the herbage are at risk of being lost from the
70 cropping system, both as gaseous emissions (NH₃, N₂O, NO and N₂) and through surface runoff or leaching of
71 nitrate (NO₃⁻) and soluble organic N (Askegaard et al., 2005; Korsæth, 2012; Larsson et al., 1998; Möller and
72 Stinner, 2009). Further, it is an expensive practice, using the land, establishing and managing the green manure
73 for a whole season with no direct income, only the expectance of higher income from future crops on the field.

74 That on-site mulched herbage contributes only slightly to the fulfilment of the following crops` nutrient
75 demand has been demonstrated in several Scandinavian field trials (Engström et al., 2007; Frøseth et al., 2008;
76 Solberg, 1995). As a consequence of herbage removal, one might expect decreased soil inorganic N availability
77 for the green manure crop, which could enhance clover and N₂ fixation and thereby compensate for the lack of
78 mulching in N pre crop effect. Hatch et al. (2007) found that removing cuttings from a grass-clover ley increased
79 fixation, compared with mulching, but Dahlin and Stenberg (2010) found no differences. Neither of these studies
80 included the N effect of these strategies on subsequent crops.

81 In spring barley, availability of inorganic N at the early tillering stage is a key factor for N uptake and
82 dry matter (DM) yield (Hauggaard-Nielsen et al., 1998). Growing spring barley, based on the nutrients from a
83 preceding green manure crop and without any additional nutrient input, is challenging under the cold Nordic
84 climatic conditions with a short growing season. Borgen et al. (2012) concluded that there is a limited potential
85 for improving N-use efficiency by management changes, in for example the time of ploughing and/or crop
86 rotation, in stockless organic cereal production systems in Norway. For more substantial improvements,
87 alternative strategies appear to be necessary. Application of digestate from green manure foliage digested
88 anaerobically in a biogas plant may be a promising option for improving yields and N recovery instead of
89 mulching (Möller and Müller, 2012; Stinner et al., 2008). In biogas plants, the easily degradable organic matter

90 is digested, releasing methane for heating or fuel and residues (digestate). The latter contain plant available
91 nutrients that may be applied as fertilizer in the subsequent season. To our knowledge, this strategy has not been
92 compared previously with other strategies for green manure management under Nordic conditions.

93 Soil structure is important for the development of the barley crop (Arvidsson, 1999), both to create good
94 conditions for root growth and for the turnover of soil organic matter (Breland and Hansen, 1996). The processes
95 and mechanisms involved in soil aggregation is complex and can be affected through management practices
96 (Bronick and Lal, 2005). Earthworm activity influences and normally improves soil structure and aggregate
97 stability (Bronick and Lal, 2005; Edwards and Lofty, 1977; Marinissen, 1994). Although earthworm species
98 have different feeding strategies, their excrements (casts) contain more plant available nutrients than does bulk
99 soil (e.g. Buck et al., 1999; Haynes et al., 2003; Pommeresche and Løes, 2009). This finding supports the idea
100 that one intensive year of “feeding” the soil with mulch material may improve soil structure and soil nutrient
101 status.

102 The effects on earthworms when green manure herbage is removed and subsequently returned as
103 digestate, instead of being mulched, have been little studied. Because the easiest available carbohydrates are
104 converted to methane and removed, less energy and organic carbon (C) will be available for earthworms and
105 other soil fauna. Ammonium and sulphide, which are toxic to earthworms (Curry, 1976) are formed by anaerobic
106 digestion. Thus mulched green manure herbage may be more favourable to earthworms than anaerobically
107 digested herbage.

108 The objective of the present work was to evaluate the effect of various strategies for green manure
109 management on the yield and N recovery of a subsequent spring barley crop, and its short term effects on soil
110 structure and earthworm populations in contrasting soils under cold climate conditions. The strategies involved
111 different options for on-site herbage management and the application of anaerobically digested green manure
112 herbage. The following hypotheses were tested:

- 113 • Removal of herbage, compared with mulching, will not affect the yield of a subsequent spring barley crop.
- 114 • Digestate applied as fertilizer for spring barley, compared with mulching the preceding season, will increase
115 the crop yield and the proportion of N input by the green manure herbage that is recovered.
- 116 • Compared to herbage removal, mulching will not increase the amount of soil N available for a subsequent
117 spring barley crop. On the contrary, digestate application will increase plant available N.
- 118 • Soil structure and earthworm populations will be negatively affected by removing the green manure herbage
119 or by one application of digestate.

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2. Materials and methods

2.1 Experimental sites, soil and weather conditions

Four field trials were established in 2008 at sites differing in soil characteristics and climatic conditions.

2.1.1 Weather and climate

The two neighbouring sites Kvithamar (63°29'N, 10°52'E) and Værnes (63°27'N, 10°57'E) share the same humid coastal climate in central Norway. Apelsvoll (60°42'N, 10°51'E) is situated inland, in eastern Norway with a drier climate and lower winter temperature. Ås (59°39'N, 10°46'E), in southeast Norway, represents an intermediate climate with respect to precipitation and winter temperature, but has the highest summer temperature of the sites. The normal values (1961-1990) for annual precipitation at Kvithamar/Værnes, Apelsvoll and Ås are 896, 600 and 785 mm, respectively, of which respectively 465, 319 and 382 mm occur during the growing season (May-September). The amounts of rainfall during the growing seasons of 2008/2009/2010 were 351/624/401 at Kvithamar/Værnes, 376/404/421 at Apelsvoll and 463/433/489 mm at Ås. The mean corresponding growing season temperatures in 2008/2009/2010 were 12.5/12.8/11.7, 12.8/13.1/12.4 and 13.4/13.8/13.1 °C, which are close to or above the normal values. During the winter prior to the barley crop (October 2009 - April 2010), the mean temperatures were -1.4, -3.2 and -1.7 at Kvithamar/Værnes, Apelsvoll and Ås. The corresponding amounts of precipitation were 534, 461 and 324 mm.

2.1.2 Soil properties

The soil at the sites is classified as a Mollic Gleysol, Arenic Fluvisol, Endostagnic Cambisol and Typic endoaqualf (IUSS Working Group WRB, 2006) for Kvithamar, Værnes, Apelsvoll and Ås. The soils at Ås and Kvithamar are derived from marine clay with relatively high silt contents, whilst that at Værnes overlies a coarse freshwater alluvium and that at Apelsvoll is developed from glacial till. The silty clay loam topsoil at Kvithamar is highly drought-resistant, but it overlies a very compact plough pan layer and compact subsoil with gley spots, both of which have low air and available water capacities. The clay loam topsoil at Ås is relatively drought-resistant and has a moderate air capacity, whereas the deeper soil layers are more compact, with low air and available moisture-holding capacities. At Værnes the soil is sandy loam and reasonably drought-resistant and well-aerated down to 0.5 m, but deeper layers have very low water-holding capacity and support little root growth. The soil at Apelsvoll is well-aerated sandy loam and relatively drought-resistant at all depths, and has

150 few physical limitations to plant growth. The deeper subsoil (> 0.6 m) is very compact. Information on the basic
151 physical properties within soil profiles at the trial sites was obtained from previous studies performed at or close
152 to the present locations (Table 1).

153 <<Table 1>> near here

154 The topsoil at Kvithamar has a high C content, whereas the content is moderate at Apelsvoll and Ås and
155 low at Værnes (Table 2). The C content in deeper horizons is very low, especially at Værnes and Ås. The level
156 of total N is considerably higher at Kvithamar than at the other sites. In general the total N level followed that of
157 C, with C/N ratios mostly in the ranges of 11-15 at Kvithamar, Værnes and Apelsvoll and 8-11 at Ås. The C and
158 N was analysed using the Dumas combustion method (Bremner and Mulvaney, 1982) on a Leco CHN 1000
159 analyzer (LECO Corp., St. Joseph, MI, USA).

160 <<Table 2>> near here

161 The soil reaction in the topsoil, measured in water, was slightly above pH 6 at all sites. The topsoil
162 contents of plant-available phosphorus and potassium were measured in 2008 by the ammonium acetate lactate
163 method (AL, 0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75, ratio of soil weight to solution volume of
164 1:20, Égner et al. (1960)) as practice in Norway. The phosphorus level (P-AL) was very high at Værnes (155 mg
165 kg⁻¹), medium at Kvithamar and Apelsvoll (66 and 75 mg kg⁻¹) and low at Ås (40 mg kg⁻¹). The potassium level
166 (K-AL) was low at Værnes (36 mg kg⁻¹) and medium at the other sites (75 mg kg⁻¹ at Ås, 89 mg kg⁻¹ at Apelsvoll
167 and 98 mg kg⁻¹ at Kvithamar).

168 At Kvithamar, the soil had been farmed organically and according to a rotation dominated by grass-
169 clover leys fertilized with animal manure from 1993 until 2003. From 2003 and until the start of the present
170 experiment, the site had been cropped with cereals with low rates of fertilization and with breaks of green
171 manure leys. At Værnes the soil had not been farmed organically. Here the crop rotations were dominated by
172 annual crops until 2000 and by grass from 2001 to 2006. In 2007 the crop was cereals. The previous rotation at
173 Apelsvoll was dominated by organically managed arable crops. In 2007 the field was fallowed with repeated
174 harrowing. At Ås the soil had been managed organically since 1993 with ley-arable crop rotation until about
175 2000, then mainly with spring cereals and undersown clover every year until 2008. Chemical weed control with
176 glyphosate was performed before ploughing in spring 2008.

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178 **2.2 Crop management and experimental treatments**

179 The green manure was a grass-clover ley established in spring as an undercrop in cereals. The year after, the ley
180 was cut several times and the herbage was chopped and left on the stubble (mulched), which is according to the
181 most common practice for management of green manure leys in Norway. In addition to mulching, the treatments
182 included removal of the herbage and application of digestate in the following year (Table 3). A control treatment
183 with repeated cereal cropping was also included. An overview of crops and management is given in Table 4.

184 <<Table 3>> near here

185 In spring 2008 the green manure plots were sown with 2 g m⁻² seed mixture consisting of 20% red
186 clover (*Trifolium pratense* L. cv. Nordi), 10% timothy (*Phleum pratense* L. cv. Grindstad), 35% meadow fescue
187 (*Festuca pratensis* L. cv. Fure) and 35% perennial ryegrass (*Lolium perenne* L. cv. Napoleon), as an undercrop
188 in unfertilized spring barley (*Hordeum vulgare* L. cv. Sunnita at 16 g m⁻²). On the control plots, unfertilized
189 spring barley was sown in pure stand. The barley straw was removed after grain harvest.

190 In 2009 the green manure leys (G) were cut three times. The chopped herbage was either mulched after
191 all cuts (mulched three times = G-3M), or removed twice and mulched after the last cut (mulched one time = G-
192 1M), or removed after all three cuts (mulched zero times = G-0M). The first cut was carried out when timothy
193 reached late stem elongation, i.e. just before inflorescences were visible on 10% of the shoots, next after 600-650
194 day degrees (base temperature 0°C) from the first cut and the third in mid-September. The stubble height of the
195 green manure was 5-8 cm. The control plots (C) were sown with unfertilized oats (*Avena sativa* L. cv. Gere,
196 Table 3). The oat straw was removed at all sites except Apelsvoll. After grain harvest in plots without undercrop
197 in 2008 and 2009, some native white clover (*Trifolium repens* L.), couch grass (*Elymus repens* L.) and
198 dicotyledonous weeds emerged. These were removed by hand weeding or frequent mowing using a small lawn
199 mower with a rotor working at the soil surface.

200 In spring 2010 the leys were ploughed under, and spring barley (*Hordeum vulgare* L. cv. Tiril at 20 g m⁻²)
201 was sown (Table 4). Prior to sowing, herbage-based digestate (D) was applied to half of the plots where green
202 manure herbage had been removed (G-0M-D), and to half of the plots with preceding oats (C-D, Table 3). The
203 digestate contained 11 g total N and 6 g ammonia N m⁻², which corresponded to about 45% of the total N
204 harvested as herbage, and in addition 1.6 g P m⁻² and 7.7 g K m⁻². It was applied on the soil surface and
205 harrowed down on the same day, or by the Direct Ground Injection (DGI) technique to 6-8 cm depth in 0.3 m
206 rows (Apelsvoll). On control plots (C-I), 8 g N, 1.5 g P and 9 g K m⁻² were applied as inorganic fertilizers. The
207 proportion of NO₃-N of the total fertilizer N, varied from 20% (Apelsvoll) to 90% (Ås).

208 <<Table 4>> near here

209 After harvesting of barley grain and straw, the fields were harrowed to control perennial weeds, except
210 at Kvithamar where such a treatment was considered to increase the growth of weeds and native clover (Table
211 4). No further management was carried out until field trials were terminated at the end of May 2011.

212 The experimental lay out was a randomized block design with 4 replicates, but the plots with the two
213 control treatments were always placed beside each other for practical reasons. The gross size of individual plots
214 depended on the implements available at each site, and varied from 48 to 60 m².

215

216 **2.3 Harvest, plant and soil sampling**

217 **2.3.1 Plant sampling**

218 Yields of barley (grain and straw separately) and of the three leys cuts were harvested with experimental plot
219 harvesters on 1.5 m x 4-5 m subplots. From the harvested grass-clover herbage (and barley straw),
220 representative subsamples were sorted manually and later dried for determination of species composition. N and
221 moisture content in the straw and herbage were determined plot-wise in other sub-samples dried at 60°C. On G-
222 3M and at the last cut of G-1M, the raw herbage was, after weighing, manually redistributed on the harvested
223 area and finely chopped with a stubble cutter.

224 Samples of the standing green manure biomass were taken as late as possible (late October) before frost
225 in 2008 and 2009 (Table 4), by cutting plants at the soil surface on 0.25 m² within each plot. In 2010 above-
226 ground biomass of barley was sampled twice early in the growing season (Table 4), by cutting two subplots of
227 0.25 m² at ground level on each plot, first at 250-330 day degrees from plant emergence (base temperature 0°C),
228 then at flag leaf sheath opening (growth stage 47 in the BBCH scale, Lancashire et al., 1991). The biomass of the
229 stubble left after harvest was recorded similarly. It was only recorded on plots receiving digestate (G-0M-D and
230 C-D), because stubble biomass is found to be relatively little affected by fertilizer treatments (Bleken, 1990).

231 Dried plant samples were finely milled (Cyclotec, mesh size 1 mm) before determination of total N
232 using the Dumas method mentioned earlier.

233 **2.3.2 Soil sampling**

234 The content of inorganic N (NO₃-N and NH₄-N) was determined in soil samples collected to 20 cm depth on 12
235 sampling dates selected for their likelihood of showing differences in mineral N fluxes (dates are given in Table
236 4). In addition, late very autumn and in spring 2010 and 2011 soil was sampled in three more layers to 80 cm
237 depth (20-30, 30-60 and 60-80). Composite sample of 6 soil cores were taken on each plot and stored frozen.
238 Before all the differences between treatments were established, samples from equally treated plots within each

239 block were pooled. Approximately 300-500 g of soil was coarsely ground without thawing and a subsample of
240 100 g was used for gravimetric determination of moisture content by drying at 105° C. Another subsample of 40
241 g was extracted with 200 ml 1M KCl, and the supernatant analysed by spectrophotometry on a FIAstar™ 5000
242 Autoanalyser (Foss Tecator AB, Höganäs, Sweden, Application Notes 5232 and 5226 (2001) for NO₃-N and
243 NH₄-N, respectively). Results were expressed on a dry weight basis and converted to area units using appropriate
244 bulk density values (Table 1).

245 Total soil C and N were analysed in samples taken at the end of the trial, as described for inorganic N.
246 Samples from treatments within the same block were pooled, ground in a mortar and analysed by the Dumas
247 method mentioned earlier.

248 In order to assess possible effects on soil structure of two years with green manure crops versus
249 continuous cereal growing, sampling was performed after ploughing and harrowing in spring 2010 soon after
250 digestate application. Five subsamples were taken by spade, altogether 5-6 litres of soil, from the seedbed down
251 to 5-6 cm depth on each plot with treatments G-3M, G-0M-D and C-D. The samples were air dried in open
252 containers at room temperature for several months before analysis.

253 Aggregate size distribution (5 groups: <2 mm, 2-6 mm, 6-10 mm, 10-20 mm, >20 mm) was found by
254 dry sieving for two minutes on a reciprocating shaker containing sieves with mesh openings of 2, 6, 10 and 20
255 mm. Stones were removed. Aggregate size groups were calculated on a weight basis and the mean weight
256 diameter was expressed using the formula of Van Bavel (1949), assuming a maximum clod size of 35 mm.

257 The stability of aggregates to simulated rainfall was measured for aggregate sizes of 2-6 and 6-10 mm,
258 using similar apparatus as that described by Njøs (1967). These aggregate size fractions accounted for 50% of
259 the total soil samples at Ås, 42% at Kvithamar, 37% at Apelsvoll and 24% at Værnes. Two subsamples (40 g) of
260 each size group were placed within a radius of 0.15 m and subjected to simulated rain for 2 minutes (pressure 1
261 bar, Hardi 4110-20 nozzles, nozzle height 0.35 m and ca. 70 passes). Aggregate stability is given as the weight
262 percentage of aggregates remaining on the sieve.

263

264 **2.4 Apparent recovery of nitrogen**

265 The apparent recovery of N in grain or above-ground biomass of barley from mulched green manure or digestate
266 was assessed by subtracting total N yield in the treatment with no mulch left behind (N yield_{G-0M}) from the total
267 N yield (N yield) in treatments receiving mulch (G-3M or G-1M) or digestate (G-0M-D), and expressed as a
268 percentage of the amounts of N applied as mulch or digestate (N applied):

269

270 Apparent N recovery (%) = $100 \times ((N \text{ yield} - N \text{ yield}_{G-0M}) / N \text{ applied})$ (1)

271

272 **2.5 Earthworm sampling and analysis**

273 Earthworms were sampled at Kvithamar and Værnes after the last cut in 2009 and after grain harvest in 2010.

274 Two soil cubes (0.2 m x 0.2 m x 0.2 m) were removed in all plots of the four treatments G-3M, G-0M, G-0M-D

275 and C-D. The earthworms were sorted from the cubes by hand. Their total biomass was recorded as g m⁻² after a

276 short storage in 75% alcohol. The density, individuals m⁻², included both juvenile and adult worms. All

277 earthworms were identified to species according to the identification key of Sims and Gerard (1999).

278

279 **2.6 Statistical analysis**

280 Analysis of variance (ANOVA) was performed using a general linear model (GLM) on soil inorganic N data,

281 plant yields, N uptake, N concentrations and clover proportion. Analyses were performed for all sampling

282 occasions for each site and in total using recordings for single plots as input data and block as random effect. For

283 the barley yields in 2008, before the different treatments took place, we tested that the variance between plots

284 was smaller than between blocks. For multiple comparisons tests, Tukey HSD procedure was used. The

285 statistical software package R was used for these calculations (R Core Team, 2012).

286 For soil aggregate data ANOVA were performed, using a split-plot design with trial site as the main

287 factor and green manure treatment as the split-plot factor (Minitab 15, Minitab Inc. State College, Pennsylvania,

288 USA). For multiple comparisons the tests LSD procedure was used. For earthworm parameters, ANOVA was

289 performed for each site and year separately, using the two samples in each plot as separate input data, and block

290 as random effect. Biomass was analysed by a linear mixed model (MIXED), while the number of earthworms

291 was analysed by the generalized linear mixed model (GLIMMIX, with negative binomial distribution and *ln* as

292 link function), both by SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA). For multiple comparisons tests,

293 Tukey procedure was used.

294 In all tests, significance was assumed at P-levels < 0.05.

295

296

297 **3. Results**

298 **3.1 Nitrogen and dry matter yields**

299 **3.1.1 Green manure and cereals, 2008-2009**

300 In the establishment year, the green manure undercrop reduced grain yield compared to the pure stand, by 22%
301 at Kvithamar, 15% at Apelsvoll and 7% at Værnes ($P = 0.058$), but no reduction was seen at Ås. At the four sites
302 the mean grain DM yield of barley with undersown green manure ranged between 194 and 262 g m⁻². The
303 standing herbage biomass and N content of the green manure in late autumn 2008 was markedly higher at the
304 two northern sites than at the others, likely caused by earlier grain harvesting at the northern sites. The biomass
305 was 297 and 240 versus 76 and 113 g DM m⁻² at Kvithamar, Værnes, Apelsvoll and Ås, respectively. The
306 corresponding N contents were 8.9 and 6.7 versus 1.7 and 3.0 g N m⁻².

307 All leys survived the winter well. In 2009, the average for the two treatments in accumulated herbage
308 biomass for three cuts was 1071, 929, 808 and 873 g DM m⁻² at Kvithamar, Værnes, Apelsvoll and Ås (Table 5).
309 Herbage removal increased the DM yield of the second and third cuts at Apelsvoll and of the second cut at
310 Kvithamar, but it did not affect the regrowth at Værnes and Ås. Similarly, herbage removal had either no effect
311 on the total amount of N at the second and third cut, or slightly increased it in the case of Apelsvoll.

312 The estimated C/N ratio in the herbage ranged between 12 and 22, based on measured N (Table 5) and
313 C contents in similar plant material analysed by Marstorp and Kirshmann (1991) and Thorup-Kristensen (1994).
314 In general, the estimated lowest C/N ratio is for the herbage in the third cut.

315 <<Table 5>> near here

316 Clover was already abundant at the first cut, and dominated over grasses at the second and third cuts
317 (Table 5). Mulching significantly reduced the proportion of clover at Apelsvoll. Similar trends were seen at
318 Kvithamar and Ås but not at Værnes. The clover biomass was significantly ($P < 0.05$) larger after herbage
319 removal for Kvithamar, Apelsvoll and Ås averaged over the three sites (data not shown). The total N yield in the
320 harvested herbage (G-0M) ranged between 19 and 26 g N m⁻², with the largest N yield at Kvithamar (Table 5).
321 The standing biomass in late autumn 2009 contained between 2.4 and 4.7 g N m⁻². Again the highest biomass
322 and N yield was observed at Kvithamar, and there was no effect of previous herbage management.

323 **3.1.2 Barley, 2010**

324 Early above-ground biomass and N content in the barley ranged the treatments approximately in the order C-I ≥
325 C-D ≥ G-0M-D ≥ G-3M > G-0M (Table 6). This indicates that green manure provided less readily available N
326 than did digestate or the use of 8 g N m⁻² fertilizer. Furthermore, removal of the herbage, rather than mulching,
327 reduced the N supply to the young barley crop. Later, at the flag leaf stage, DM and N content still followed the
328 same pattern. At Apelsvoll, however, digestate had a less positive effect on the young barley crop than at the

329 other sites, with no differences between G-0M and G-0M-D. The biomass and especially the N uptake was
330 higher on the sandy soil at Værnes than on the clay soil at Kvithamar, and the differences increased from the first
331 to the second sampling date, in spite of the facts that the N yield of the green manure had been somewhat higher
332 at Kvithamar (Table 5) and that the two sites were exposed to the same cold weather in early summer 2010.
333 Early in the growing season, chlorosis typical of N deficiency was observed in the treatments where the herbage
334 had been removed the previous year (G-0M and G-1M), particularly on the two clay soils (Kvithamar and Ås). It
335 was most severe at Kvithamar, where the average soil temperature at 10 cm depth was 10.6°C from plant
336 emergence to 1st sampling, compared to 13.3°C at Ås.

337 <<Table 6>> near here

338 In order to explore the N state of the young plants we plotted them against published critical and
339 minimum N dilution curves for winter and spring wheat (Justes et al., 1994; Ziadi et al., 2010). In all cases N
340 concentration was far below the critical dilution curves, and very close or even below the minimum curves (Fig.
341 1), particularly in the case of plots where the green manure herbage had been removed (G-0M).

342 <<Fig. 1>> near here

343 Barley grain yields in 2010 were close to the national average for conventional farming, about 300 g
344 DM m⁻², except at Kvithamar, where the yield was only half of that. The barley crop there was particularly low
345 on one of the blocks, apparently due to poor soil structure. At Ås, high precipitation after ripening delayed
346 harvesting and reduced the recovered yield, especially on the most productive plots, where up to 90% lodging
347 occurred and at least 10% of the ears remained on the ground after harvest. No correction was made for these
348 losses.

349 At all sites there was a consistent trend for grain DM and N yields in the order C-I ≥ G-0M-D ≥ G-3M ≥
350 C-D ≥ G-1M ≥ G-0M (Table 7). Relative to the early growth stage, the ranking of the G-3M and G-0M-D
351 treatments had improved, but that of C-D was less good. N uptake after growth stage 47 up to maturity was
352 lowest in treatment C-D and highest on treatment G-0M-D (1.8 and 4.6 g N m⁻², respectively, averaged over all
353 sites). Furthermore, C-D had the lowest N harvest index, i.e. proportion of N in grain relative to the total N in the
354 above-ground biomass (data not shown). The mulching of even a single cut (G-1M) improved grain DM and N
355 yields, compared to G-0M, and mulching three times raised them evidently (Table 7). At site level, the latter was
356 significant on the two sites with sandy loam (Værnes and Apelsvoll). The increased N yield in grain caused by
357 previous green manure stubble and roots, as seen by comparing G-0M-D and C-D, ranged from 0.1 to 1.8 g N m⁻²
358 (Table 7).

359 <<Table 7 >> near here

360 The N concentration of the grain was higher after green manure than in C-I at the two northern sites
361 (Kvithamar and Værnes), and a similar tendency was present at Ås (Table 7). This indicates that mineralization
362 of green manure residues during late summer contributed positively to grain protein. At Kvithamar and Værnes,
363 application of digestate on green manure (G-0M-D) diluted the grain N% to the same level as that of the C-I
364 plots.

365 The biomass of the barley stubble, found in G-0M-D, and used for the calculations for the apparent N
366 recovery, contained 0.46, 0.69, 0.26 and 0.28 g N m⁻² for Kvithamar, Værnes, Apelsvoll and Ås.

367

368 3.2 Apparent recovery of nitrogen

369 The apparent N recovery from mulched green manure herbage or digestate in above-ground barley
370 biomass or grain was low and in the order of G-0M-D > G-1M > G-3M (Table 8). The ranking reflects the level
371 of N input to the systems, but also the lower N yield of G-1M relative to the other treatments.

372 <<Table 8>> near here

373

374 3.3 Inorganic nitrogen in soil

375 No high levels of inorganic N caused by mulching were found in either the top-soil (Fig. 2) or in deeper soil
376 layers (data not shown).

377 <<Fig. 2 >> near here

378 In spring 2009, one year after the green manure ley was established, the level of NO₃-N in the soil was
379 lower in plots with green manure than in the control plots with barley stubble only, at all sites except Kvithamar
380 (Fig. 2).

381 Ten and twenty days after the first cut, there were no significant differences in inorganic N in the soil
382 layer 0-20 cm, whether or not the green manure herbage had been mulched.

383 At all sites, the NO₃-N or inorganic N in soil in late autumn 2009 was higher with G-3M than with G-
384 0M, but both levels were below 1 g m⁻². From late autumn 2009 until spring 2010, the level of inorganic N at 0-
385 20 cm increased in all treatments by 0.4-1.0 g N m⁻². The temperature at 10 cm depth after soil sampling in
386 autumn was on average 2°C for 1.5-2 months, then below freezing for 3.5 months and finally 2-4°C for 10-20
387 days before sampling in spring.

388 In spring 2010, before the green manure was ploughed under, there was at all sites a higher level of
389 inorganic N in soil with mulched green manure (G-3M), compared to the other treatments. However, 3-5 weeks
390 after ploughing, two weeks after germination of the barley crop, there were no differences in the levels of
391 inorganic-N in the top-soil between treatments G-3M and G-0M. Application of digestate (G-0M-D) tended
392 (P=0.057) to enhance the amount of inorganic N in the top-soil at Apelsvoll compared to mulching. No such
393 differences at the other sites, and in general a lower soil inorganic N content, may be a consequence of higher
394 precipitation from digestate application to soil sampling at these sites (45-78 mm) than at Apelsvoll (8 mm).

395 After the barley crop, there was no significant difference in the level of soil inorganic N related to
396 herbage management, but soil inorganic N was in general higher with previous green manure than without,
397 though in most comparisons at site level it was not statistically significant.

398

399 **3.4 Soil aggregate size distribution and aggregate stability**

400 There were significant overall differences between treatments on the stability of both aggregate size groups (2-6
401 and 6-10 mm), with no significant interaction with site (Table 9). Treatment G-0M-D gave greater stability than
402 did treatments G-3M and C-D, whilst the latter had in most cases similar stability. Since there was no overall
403 effect of treatment on any of the aggregate size fractions, these results are not presented.

404 <<Table 9>> near here

405

406 **3.5 Earthworms**

407 In general, higher densities and biomass of earthworms were found in G-3M, where the green manure was
408 mulched and left on the soil surface, than in treatments where it was removed (G-0M, G-0M-D) or where only
409 cereals were grown (C-D) (Table 10, 2009). This difference was also seen in autumn 2010, but only for the
410 number of worms in the clay soil at Kvithamar (Table 10). No significant effects of digestate were found, when
411 comparing treatment G-0M-D and C-D with G-0M in 2010 (Table 10).

412 <<Table 10>> near here

413 Fieldworm (*Aporrectodea caliginosa*) was the main species found in both soils, 63% of the individuals
414 at Kvithamar (K) and 86% at Værnes (V). Some *Lumbricus rubellus* (15% (K) and 11% (V)) and *Aporrectodea*
415 *rosea* (14%) were found, the latter only at Kvithamar, as well as a few specimens of *Lumbricus terrestris*. More
416 *L. rubellus* (P < 0.01) were found in treatment G-3M at Værnes in 2009 than in the other three treatments
417 (species data not shown). This effect was not seen in 2010. At Kvithamar, in the clay soil, more *A. caliginosa*

418 were found in the same treatment (G-3M) only in 2010 ($P < 0.01$). No effects of digestate were found on the
419 species composition, when comparing treatment G-0M-D and C-D with G-0M in 2010.

420

421 **4 Discussion**

422 **4.1 Effect on barley yield of removal versus mulching of herbage**

423 Contrary to the hypothesis, we found that removing green manure herbage compared to mulching affected the
424 subsequent spring barley yields negatively. Removal of herbage increased N-deficiency in the following barley
425 crop (Fig. 1) at all sites, and this was reflected in a consistent trend with substantial and statistically significant
426 yield loss at harvest on two of the sites. On the sandy and loam soils, mulching increased the grain yield by 23
427 and 33%. This is comparable to the 20% yield increase of spring barley following vegetables mulched with a
428 chopped grass-clover mixture on a nearby morainic loam, reported by Riley et al. (2003).

429 In the case of the clay soils, results varied. At Kvithamar, with colder weather during first part of the
430 season (on average 2°C), all treatments were severely N deficient and the yield considerably reduced, but the
431 relative difference between the treatments were still high. At Ås, barley grew well, but likely differences
432 between treatments were lost due to late season lodging caused by wet weather conditions after ripening. This
433 delayed harvesting, and led to loss of grain. From visually observations we anticipate that the grain losses were
434 greatest on the plots with best growth.

435 We expected higher clover proportion in the green manure ley caused by removal of herbage, and a
436 positive effect of this on N availability for barley the following year. However, only at Apelsvoll the removal of
437 herbage facilitated larger regrowth and proportion of clover. Even at Apelsvoll, the larger clover content did not
438 result in larger availability of N the year after. In general, no effect of mulch for grass and clover regrowth may
439 indicate that N is either lost or immobilized.

440 The C/N ratio is found to be the most important factor determining the mineralisation from fresh plant
441 material (Thorup-Kristensen, 1994; Marstorp and Kirshmann, 1991). Net immobilization of N is likely since the
442 estimated average C/N ratio of the grass-clover herbage is above 15, which is found by Marstorp and Kirshmann
443 (1991) to be a turning point for legumes. Furthermore, high herbage yields overlying and shading the stubble
444 may also have suppressed the ley regrowth.

445 The decomposition rate of plant material and N mineralization from soil organic matter have both been
446 found to be slower in clay soils than in sandy soils (Hassink et al., 1993). Shah et al. (2013) found that the total
447 plant N recovery of applied manure in ryegrass followed the same pattern as above with regard to soil type. The

448 limited fertilization effect of mulching in our study on the clay soils, despite signs of N-deficiency in barley in
449 G-0M, may have been caused by slower mineralization; an effect of soil type, especially in combination with
450 cold and wet weather conditions as for the site Kvithamar. The in general low estimated fertilizer N recovery in
451 the barley grain, and especially for Kvithamar, indicates poor growth conditions for the barley plants in early
452 growing season.

453 The progress and the level of N loss by leaching, runoff and gaseous emissions from the mulched
454 herbage are strongly influenced by the weather conditions. Ammonia emission is more affected by precipitation
455 than temperature or N-concentration in the herbage (Whitehead et al. 1988). Whitehead and Lockyer (1989)
456 found that decomposing grass herbage placed on the stubble of a cut sward containing 3% N, lost 10% of its N
457 as ammonia during 28 days with showery weather. However, emission levels twice as high as this, or even up to
458 39%, have also been found from grass with lower N content (Larsson et al., 1998; Whitehead et al., 1988).

459 At the Ås site, Nadeem et al. (2012) observed that mulching of the herbage only increased nitrous oxide
460 (N₂O) emissions slightly. In the year with green manure, the emission was 0.037 g N₂O-N m⁻² higher throughout
461 the whole growing season than where herbage was removed. Some ammonia and N₂O emissions are likely to
462 have occurred, especially after the second and third cuts at the two Northern sites, due to precipitation of 100-
463 250 mm during the first 30 days after the cuts.

464

465 **4.2 Effect on barley yield of digestate versus mulched herbage**

466 When half of the N in green manure herbage was applied as biogas digestate in spring, the barley DM yields
467 reached the same level as after mulching the herbage, and the apparent N recovery was higher, as hypothesized.
468 The recovery in spring barley grains of NH₄-N applied in digestate on the two sites with the lightest soils,
469 Værnes and Apelsvoll, was similar to the 29-38% recovery from manure (slurry) found by Olesen et al. (2007).
470 In general, the digestate appeared to contribute more to the nutrient supply during early growth than did N
471 mineralization from the green manure ley. The latter, contributed mainly later in the growing season and thus
472 increased protein content more than the biomass. The low N harvest index in C-D indicates that an adequate
473 early N supply to the crop was followed by a period of more severe N deficiency.

474 The low DM levels in the early growth stages of barley fertilized with digestate at Apelsvoll may have
475 been a result of the DGI application technique. The row-spacing of the digestate applied with DGI was relatively
476 large compared to the plant rows.

477

488 **4.3 Effect of herbage management on plant available nitrogen in soil**

489 Green manure herbage, if mulched, represents a high N input to the cropping system, but no high pulse of
490 inorganic N caused by mulching was found in the soil on the sampling dates.

481 The latest measurements of soil inorganic N before barley crop nutrient uptake, 7-10 days after
482 germination, showed a consistent trend in the order $G-0M-D \geq G-3M \geq G-0M$. Overall, only digestate
483 application enhanced significantly the the level of plant available N in soil compared to previous removal of
484 herbage. This is in according to the hypothesis that plant available N will increase after digestate application, but
485 not after mulching. However, in the case of mulching versus removal, on the soils most prone to leaching, we
486 found a substantial yield response in barley from the mulched herbage. In accordance with the findings of Dahlin
487 et al. (2011), this indicates that a considerable proportion of herbage N was incorporated into soil organic matter
488 and mineralized during the growing season 2010.

489 Thorup-Kristensen and Dresbøll (2010) have recorded fast N mineralization under low temperatures
490 after incorporation of catch crops in spring. Our results from N uptake in the barley plants indicates a slower N
491 mineralisation from the ploughed under one year old mulched green manure ley, probably due to a higher C/N
492 ratio of the plant material. The contribution of N from green manure root and stubble to the following grain yield
493 was low. In general, higher levels of NO_3-N were found in the G treatments than in the C treatments in spring
494 2011. This indicates that N from green manure was released over a longer period.

495 Some mineralization in soil with and without green manure seems to have taken place during the winter
496 of 2009 to 2010 (Fig. 2, comparing bars for inorganic N in autumn 2009 and spring 2010). This is in agreement
497 with several studies that have shown substantial mineralisation of incorporated green manure at temperatures
498 down to 1-3°C (Cookson et al., 2002; Magid et al., 2001; Van Schöll et al., 1997). Our results also indicate that
499 mineralization from stubble and below-ground green manure biomass occurs at low temperatures. The enhanced
500 soil inorganic N content due to mulching that was found before ploughing the ley in spring 2010, corresponded
501 to 4-7% of the total N added as mulch.

502

503 **4.4 Effect of herbage management on soil structure and earthworms**

504 The use of digestate improved soil aggregate stability, more than compensating for the herbage removal
505 in treatment G-0M-D, as seen in the comparison to treatment G-3M. Further, it would seem that the use of
506 digestate was effective in increasing the soil aggregate stability on plots previously cropped with cereals to the
507 level found where mulching of green manure had been practiced. As found by Abvien et al. (2009), easily

508 decomposable products are known to have an intense and transient effect on soil aggregate stability. Further,
509 digestate of cattle slurry is found to stimulate the bacterial decomposer community more than undigested slurry,
510 and in a similar way to that of inorganic fertilizers (Walsh et al., 2012).

511 In accordance to our hypothesis, a clear positive effect of mulching on the earthworm fauna was seen
512 after only one season. It is well-known that systems with one or more years of grass and clover in the crop
513 rotation, often host more earthworms than do all-arable systems (Edwards and Lofty, 1977; Pommeresche and
514 Løes, 2009; Schmidt et al., 2003). In our study, the effect was consistent even the first year and was directly
515 connected to the mulching of the plant material. One result of higher biomass of earthworms after mulching in
516 2009 was higher cast production. Pommeresche and Løes (2009) estimated that a density of 229 earthworms m⁻²
517 corresponds to 22.1 kg casts per m⁻² year⁻¹. The casts contained 3 g total N kg⁻¹. Roughly estimated for our trials,
518 this means 30 g more total N m⁻² in casts in the mulched treatment. Almost the entire N content of the casts is
519 organically bound and thereby protected against leaching until mineralization (Boström, 1988). In addition, dead
520 earthworms contributes to the N mineralization as their body tissue contains 10-12% N of their dry weight and
521 they decompose rapidly (Edwards and Bohlen, 1996).

522 The higher densities of *L. rubellus* and *A. caliginosa* after mulching are a response to the input of
523 organic matter which increased both the survival of adults and juvenile recruitment. Among species, mulching
524 gave diverging results in the two soils. At Værnes, the surface dwelling *L. rubellus* responded by increased
525 population in the same season. Slower decomposition in the denser clay soil at Kvithamar, may explain the
526 higher density of the soil-dwelling, soil-eating *A. caliginosa* here in 2010, compared to the lack of any effect on
527 the earthworm parameters at Værnes in the same year.

528 The lack of any effects on the earthworm density or biomass after one digestate application is not in line
529 with the hypothesis. However, one season is too short a time to conclude on possible longer term effects on
530 earthworm parameters that may occur with repeated annual use of digestate. Ernst et al. (2008) tested effects on
531 earthworms of cattle slurry and anaerobic digested mixture of cattle slurry and plant residue in a microcosm
532 experiment. While the biomass of the litter-eating species (*L. terrestris* and *Apporectodea longa*) increased in
533 both slurry treatments, that of the soil-eating species *A. caliginosa* decreased significantly in treatments with
534 digested slurry. This is unfortunate as soil-eating species survive well with soil tillage and are the dominant
535 species (80-100%) in arable soils in Norway (Chan, 2001; Pommeresche and Løes, 2009).

536

537 **4.5 Implications**

538 Our results indicate that in spring barley production under cold climatic conditions, the N supply may be
539 limiting, even after a productive green manure ley. If the green manure herbage is removed, it can lead to further
540 N deficiency. Mulching the last cut and removing the previous cuts will reduce the potential loss of N from the
541 cut herbage, compared to mulching all cuts, but the subsequent barley DM yield may be also be lower.

542 Removing herbage from the field requires that it may be used as forage or to produce digestate in a
543 biogas reactor. Möller and Müller (2012) concluded that biogas digestion of field residues, instead of mulching,
544 resulted in a win-win situation, with additional energy yields, lower risk of N leaching and lower nitrous oxide
545 emissions, although the risk of ammonia volatilization remains when applying the digestate. Halberg et al.
546 (2008) also support this view from an energy self-reliance perspective on organic cash-crop farms.

547 In our trial, we applied nearly half of the N harvested in green manure herbage. At a farm scale, the
548 surplus digestate would make it possible to fertilize other fields as well. However, running small farm-scale
549 biogas plants solely based on green manure herbage is challenging. Cooperation with biogas plants with
550 continuous feeding throughout the year would seem to be the best solution until new technology is developed for
551 small farm-scale biogas plants based on grass/clover herbage.

552

553 **5. Conclusions**

554 When we evaluated the effect of various strategies for green manure management we found that both for DM
555 yield and apparent N recovery in a subsequent barley crop, it mattered how the green manure herbage was
556 managed. Herbage mulching compared to removal improved the barley yield, whilst herbage removal
557 accompanied with return of about 45% of the removed N as digestate improved both yield and N recovery. The
558 amount of N removed with the herbage was not compensated for by increased clover growth in the summer
559 regrowth. The low effect of green manure on N supply to the following grain crop was most likely due to low net
560 N mineralization. Lowest N recovery was found on the clay soils.

561 The mulched plant material gave an increase in earthworm density and biomass. Application of
562 digestate increased aggregate stability measured shortly after application, but did not affect the biomass or
563 density of earthworms.

564 Of the managements considered, the digestate strategy seems to be the most promising option as regards
565 increasing N recovery and reducing the risk of N losses.

566

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573

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