IMPACT OF THE QUALITY OF ORGANIC AMENDMENTS ON SIZE AND COMPOSITION OF THE WEED SEED BANK

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Running head: Effect of fertilizer quality on weed seed bank

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Summary

In addition to improving the soil quality, organic amendments of soils may affect weed seed survival, emergence, growth and reproduction. This study evaluated the effects of applications of different qualities of organic amendments on size and composition of the weed seed bank in a field under sequential cropping over four years. Fertilisation systems tested included: farmyard manure, vegetable fruit and garden waste compost, two types of farm compost differing in carbon:nitrogen (C:N) ratio, cattle slurry and mineral fertiliser. All organically amended plots received equal amounts of C. Crop growth was equalised on all plots by applying supplemental mineral N. Seed bank sampling took place in May 2009 to a depth of 10 cm. The weed seed bank was analysed with the seedling emergence method. Despite equal crop growth in fertilised plots, total seed bank density was lowest in plots amended with compost with low C:N ratio and highest in slurry amended plots. Observed differences in seed bank densities reflected differences in soil organic carbon content and microbial biomass. At plot level, hard-coated seeds in the seed bank (e.g. *Chenopodium* spp.*)* were inversely related to soil microbial activity. Observed differential responses of species to applied fertilisers might be attributed to interspecific differences in resistance against microbial seed degradation. Compost based fertilisation systems could be sustainable tools for incorporation into integrated weed control strategies aiming at depleting the weed seed bank.

Keywords : Microbial biomass, fertilizer quality, compost, animal slurry, mineral N fertiliser, weed suppression, PLFA

Introduction

Depletion of the soil seed bank is critically important in overcoming yearly weed infestations (Aldrich, 1984). Besides the prevention of seed return, a successful management system aimed at the depletion of the seed bank should also increase the seed mortality and manipulate weed germination and emergence (Riemens *et al*., 2007). Weed seed persistence in soil seed banks is thought to be determined by a combination of factors, including heritable traits, the maternal environment in which a seed develops, as well as soil biological, chemical, and physical properties (Gallagher & Fuerst, 2005).

The addition of soil organic matter (SOM) changes nitrogen (N) and carbon (C) turnover and soil microbiota, which may influence seed mortality, seed vigour and germination. Weed seed mortality rate, together with weed seed germination, determine soil seed bank depletion rate. The main mortality factors of seeds in the seed bank are natural physiological ageing, predation and attack by bacterial and fungal microorganisms. The relative importance of these mechanisms varies with species and environmental conditions. Biological activity (Kremer & Li, 2003) and fungal colonisation of seeds (Pitty *et al*., 1987) in the soil are positively linked with SOM. Organic matter amendments may increase soil microbial biomass and activity (Fraser *et al*, 1988) and change the incidence and severity of soil-borne diseases of weeds (Conklin *et al*., 2002). Decomposability and nutrient availability of organic amendments will influence the composition of the soil biota, responsible for the breakdown or mineralisation of the applied organic matter. Microbial decomposition of organic matter is driven by the (chemical) composition of the organic matter (e.g. the C:N ratio)(Jensen *et al*., 2005). Raw manures, slurries and sewage sludge (low C:N ratio and hence high nutrient (N) content) are mainly considered as nutrient suppliers, while stable organic amendments, like compost, add to SOM and improve soil structure. Weed seed germination and early growth is triggered by various factors, including soil temperature, soil moisture, light and soil nutrient concentrations (Karssen & Hilhorst, 1992). In particular, mineral NO_3-N and the timing of its application is known to stimulate germination of many

weed seeds (Baskin & Baskin, 1998; Sweeney *et al*., 2008). The chilling or light requirement for seed germination in some species can be replaced by N, particularly nitrate (Egley & Duke, 1985).

Kennedy and Kremer (1996) hypothesised that the soil environment could be manipulated to create "weed-suppressive soils" in which the microbial community composition and activity deplete the weed seed bank, reduce possibilities of weed seedling establishment and reduce weed growth and competitive ability. Such soils might be created by the addition of manures and composts. The lack of knowledge about the impact of the quality of the organic amendments on microbes that degrade weed seeds or weed seedlings make the hypothesis prone to criticism.

The objective of this study was to evaluate the effects of continuous application of six different fertilisation systems on weed seed bank density and composition. Furthermore, the relationships between weed seed bank density, soil organic carbon content and microbial biomass were explored. Fertilisation systems tested included continuous application of one pure synthetic fertiliser and five organic fertilisers used in Belgian agriculture (i.e. three compost forms, animal slurry, farmyard manure). Organic fertilisation systems differed in the quality (e.g. C:N ratio) of the applied organic matter but not in quantity of applied organic carbon.

Materials and methods

Field study

A long-term field experiment was set up in 2005 at the experimental farm of Ghent University at Melle (Belgium, 50°59'N, 03°49'E, 11m above sea level). The field experiment was established on a sandy loam soil with 11.7% clay, 52.0% loam and 36.3% sand. Initial soil chemical properties of the field (0-20 cm) were: organic carbon 1.01%, total N 0.086% and pH-KCl = 5.90. Average annual rainfall (over 30 years) for this area was 718 mm and average minimum and maximum air temperature was 5.6°C and 13.5°C, respectively. Prior

to the experimental period, the study site was continuously cropped with minerally fertilised maize for 22 years. During the experimental period, from 2005 until 2009, the field was subsequently cropped with fodder beet (*Beta vulgaris* L.), winter wheat (*Triticum aestivum* L.), red cabbage (*Brassica oleracea* L. var. *rubra*), perennial ryegrass (*Lolium perenne* L.) and maize (*Zea mays* L.). After the harvest of the winter wheat, phacelia (*Phacelia tanacetifolia* Benth.) was sown as a catch crop.

The field experiment was a randomised complete block design with four replicates comparing six fertilisation systems: farmyard manure (FYM), vegetable fruit and garden waste compost (VFG), two types of farm compost differing in C:N ratio (CMC1 and CMC2), cattle slurry (CSL) and mineral fertiliser (MIN N). All amendments were supplied before sowing or planting. All plots were 8 x 6 m and arranged contiguously. Due to the use of a microbial starter, which is added at the beginning of the composting process, farm compost is often called CMC compost in which CMC stands for “controlled microbial composting”. CMC1 was composed of C-rich, woody material resulting in a final C:N ratio of ca. 20-40. CMC2 was particularly made from green, N-rich materials and had a final C:N ratio of 10-20. Based on the difference in starting materials and C:N ratio, CMC1 is generally believed to be more fungi-dominated, while CMC2 is presumed to be more bacteria-dominated.

Fertiliser systems were scheduled in such a way that all organically amended plots received equal amounts of organic C, equal amount of plant available N during the growing season and equal minimum levels of K and P, allowing a comparable crop performance. By using this design, differences in seed bank composition and density can reasonably be attributed to the type or quality of organic fertiliser. Amounts of organic C applied varied from 1101 to 4000 kg ha^{-1} (Table 1). Initial doses were quite high, in order to speed up the appearance of possible effects of the organic amendments. Perennial ryegrass received a smaller quantity because it is known to build up much SOM by roots and stubbles. The catch crop received less SOM than main crops. On the CSL plots, part of the organic C was applied as crop residues (except before phacelia, red cabbage, perennial ryegrass and maize) to avoid the input of an excessive amount of mineral N. At each amendment, extra

mineral N (ammonium nitrate 27% N) was applied, in order to equalise plant available N (Table 1). Applied amounts were based on the mineral N (NO_3^- and NH_4^+) present in the soil at the time of fertilisation and the (potential) mineralisation rates of the soil and of the organic amendments, both determined by laboratory incubation (De Neve & Hofman, 1996). Except for the winter wheat, slurry-amended plots did not receive extra mineral N, since about 55 % of the N contained in cattle slurry was in mineral form. At each fertiliser application, plots were supplemented with muriate of potash 40% and triple superphosphate 45% to achieve equal levels of plant available K_2O (300 kg ha^{-1}) and P_2O_5 (100 kg ha^{-1}). Prior to sowing or planting, organic amendments, as well as mineral N fertiliser, were applied manually on cultivated and rotary harrowed plots. Organic amendments were incorporated to a depth of 20 cm using a rotary tiller when preparing the seed-bed. Rotary tillage was preferred over ploughing, in order to minimise horizontal transfer of fertilisers, seeds or microorganisms. All mentioned tillage operations were performed on all plots including MIN N plots.

Table 1 near here

Cropland was placed under conventional pest management. Sowing and planting dates and pesticide applications from 2005 to 2009 were presented in Table 2.

Table 2 near here

Seed bank sampling

Seed banks were sampled on 13 May 2009, after sowing, in the central area (6 x 4 m) of each plot, to avoid seed transfer from adjacent plots by rotary tillage operation. Within this central area 24 soil cores of 0-10 cm depth were taken on the intersections of a 1 x1 m grid with a 4.0 cm diameter steel probe. The 24 soil cores from each area were combined to form one bulked sample. Each bulked sample was further split into three subsamples. All samples

were stored at 4℃ for two weeks in darkness, before being washed within one week consecutively through 4 mm and 0.2 mm sieves. All residues passing through the 4 mm sieve but not through the 0.2 mm sieve were recovered and air dried for 3 days in a glasshouse. The seedling emergence method was used to quantify seed density. Plastic trays, 45 x 45 cm, were filled with a 2 cm layer of porous clay granules (Argex), covered by a 4 cm thick layer of sterilised peat. On top of this peat layer, the air-dried residue was spread out evenly in a 1 mm layer and covered with a 1-2 mm layer of sterilised sieved (2 mm mesh) peat. Concentrating the samples by wet sieving and using thin layers in the germination trays ensured that all seeds were exposed to light and suitable temperatures The plastic trays were kept for 12 months (1 June 2009- 15 June 2010) in a semi-open tunnel under a fine-mesh gauze cover to avoid contamination by wind-borne seeds. Optimum moisture conditions in the trays were maintained by regular sub-irrigation, except for a two-week drought period imposed in August 2009 to break seed dormancy. At the end of the drought period, trays were stirred and sub-irrigation was reactivated. The lowest night-temperature in the tunnel was -14℃ and the maximum day-time temperature was 33℃. Emerged seedlings were periodically identified, counted and removed from the plastic trays. Seedling identification was based on Hanf (1982). Nomenclature of species follows Van der Meijden (2005). Owing to the combined effect of 1) dormancy breaking activities, such as cold storage in refrigerator, dry periods, stirring, overwintering, leaching out of germination inhibitors by washing with running water and scarification of the seeds on the sieves, 2) thin seed layers in germination trays and 3) the long screening period, the measured active seed bank closely reflected total viable seed bank. This was affirmed by squeezing the non germinated seeds recovered from two randomly chosen test trays: only 1% of the larger seeds remained firm when squeezed with forceps.

Weed seed bank density was calculated as the number of seedlings in the sampled soil volume (=3.01 L) divided by the total surface area of 24 soil cores (=0.0301 m^2, i.e. surface area of the top of the core multiplied by 24) and finally expressed as the number of seedlings per m^2 to a depth of 10 cm. Total weed seed bank density was defined as the sum

183 of weed seed bank densities of all species. Relative density was calculated as the total
184 number of seedlings for a given species, divided by the total number of seedlings. Seed bank
185 numbers reflect germinable seeds, i.e. non-dormant seeds or seeds released from dormancy
186 during the seed bank screening period in the gauze tunnel.
187
188 *Seed content in organic fertilisers*
189 In order to take account of potential weed seed input from organic fertilisers, all organic
190 fertilisers were analysed for their content of germinable seeds. For each organic fertiliser
191 applied in 2009 and CSL and VFG applied in 2008, four random samples of 2 kg were taken
192 after mixing. Samples were washed through 0.2 mm sieves. The residue collected on the
193 sieve was further analysed for germinable seeds with the seedling emergence method
194 described above.
195
196 *Crop dry matter (DM) yield*
197 In order to reasonably attribute possible differences in seed bank composition and density to
198 fertiliser type or quality, crop biomass should be equal. This was checked by harvesting all
199 crop plants in the central 2 x 4 m of each plot. Fresh biomass samples were taken per plot,
200 chopped and dried for 12 h at 75℃ to calculate abo veground dry matter (DM) yield.
201
202 *Microbial biomass and composition and soil organic carbon content*
203 Analysis of phospholipid fatty acids (PLFAs) was performed, in order to explore relationships
204 between weed seed bank density and microbial biomass. PLFAs are essential membrane
205 components of all living cells and make up a relatively constant proportion of the biomass of
206 organisms. Owing to their rapid degradation after cell death, PLFAs are reliable measures of
207 the viable cell biomass. Preparation of PLFAs followed the modified Blight and Dyer
208 technique described by Balser (2001) and consisted of three steps, i.e. the extraction of the
209 lipids, the isolation of phospholipids and the methanolysis of these phospholipids resulting in
210 fatty acid methyl esters (FAMEs). These FAMEs were finally analysed by gas

chromatograph-mass spectrometer analysis. The dataset of all fatty acids was further simplified by using marker fatty acids of selected microbial groups following Kozdroj and van Elsas (2001). For Gram-positive bacteria, the sum of *i*C15:0, *a*C15:0, *i*C16:0, *i*C17:0 and *a*C17:0 was used. The fatty acid *cy*C17:0 was considered to be typical for Gram-negative bacteria, while for the actinomycetes, the sum of the *10Me* fatty acids was regarded as a reliable indicator. The C18:2*ω*6,9c was used as a signature fatty acid for fungi. One bulked sample per plot was analysed for PLFAs. Each bulked sample comprised 15 soil cores of 0-10cm depth taken in September 2007 in the central area (6 x 4 m) of each plot. In autumn 2008, soil organic C content was measured by dry combustion at 1050℃ using a TOC-analyzer (Skalar). The pH was measured potentiometrically in a 1:2.5 soil:KCl extract.

Statistical analysis

Weed seed bank densities and weed emergence were fourth-root transformed to meet the assumptions for homogeneity of variance and normality. SPSS15.0 for Windows was used to carry out the statistical computations for analysis of variance of a randomised complete block design, for linear correlation and regression analysis. Differences between treatment means were compared using Fisher's protected LSD test at the 5% significance level.

Analysis of the weed community composition was performed on arcsin-transformed data of species relative density. The linear techniques Principal Components Analysis (PCA) and Redundancy Analysis were used to analyse the weed seed bank composition (utilising Canoco 4.5), because the gradients were short (<2 SED) (Ter Braak & Smilauer, 1998). Fertilisation systems (nominal variables) were included as dummy variables and inserted as environmental variables in an indirect gradient analysis (RDA). The four replicates were inserted as covariables. Significance of the eigenvalues (λ) of the RDA ordination axes was calculated using a permutation test. RDA followed by Monte-Carlo permutation test was used to calculate the amount of variance in the species data explained by each treatment and its statistical significance (Ter Braak & Smilauer, 1998).

Results

Seed content in organic fertiliser

Organic fertilisers, applied in 2009, contained on average 0.0, 2.5, 15.1, 1.2 and 3.3 viable seeds per kg for VFG, CMC1, CMC2, CSL and FYM, respectively. So, taking into account their applied amounts (Table 1), soil seed banks were enriched with 0.0, 6.3, 58.2, 11.5 and 16.7 germinable seeds per m² to a depth of 20 cm for VFG, CMC1, CMC2, CSL and FYM, respectively. VFG and animal slurry applied in 2008 contained on average 0.0 and 2.7 viable seeds per kg, respectively. This corresponded to a seed input of 0.0 and 8.1 germinable seeds per m² to a depth of 20 cm for VFG and CSL, respectively.

Weed seed bank density

In total, 32 species were recorded. Altogether they accounted for 98.4% of the total weed seed bank density. Majors species contributing $\geq$ 0.5 % to the total weed seed bank are listed in Table 3.

Table 3 near here

Fertilisation system significantly affected total weed seed bank density (Table 3). Total weed seed bank density was lowest in compost amended plots (CMC1, CMC2 and VFG) and highest in slurry amended plots. Total weed seed bank density in minerally fertilised plots was not significantly different from seed bank densities in organically amended plots. Within organically amended plots, total weed seed bank density was significantly lower in plots receiving VFG and CMC2 compost than in slurry amended plots.

The fertilisation system significantly affected weed seed bank density of *Capsella bursa-pastoris* (L.) Medik., *Cerastium glomeratum* Thuill., *Chenopodium album* L., *Chenopodium polyspermum* L., *Lamium purpureum* L. *Plantago major* subsp. *major* L., *Polygonum aviculare* L. and *Stellaria media* L., but had no effect on *Cardamine hirsuta* L.,

Gnaphalium uliginosum L*., Poa annua* L., *Polygonum maculosa* Gray, *Senecio vulgaris* L*.* and *Solanum nigrum* L*.* (Table 3). Compared with CSL plots, MIN N plots showed significantly lower seed density of *S. media.* Plots amended with CSL showed significantly higher seed densities of C*. bursa-pastoris, C. album*, and *P. major* subsp. *major* compared with CMC2 plots and higher densities of *L. purpureum* and *S. media* compared with VFG plots. CMC2 plots showed significantly lower seed density of *C. album* than CMC1, CSL and MIN N plots*.* VFG plots showed significantly lower seed density of *L. purpureum* than CMC1 plots. MIN N and CSL plots showed significantly lower seed densities of *P. aviculare* than FYM and CMC1 plots*.* Within compost plots, seed density of *C. album* was significantly higher for CMC1 plots.

Weed seed bank composition

The first two ordination axes of the RDA (λ= 0.20 and 0.11 respectively) were significant (*P*≤0.002). Replicates were responsible for 27% of the variance in species data, whereas treatments explained 18% of the variance. MIN N, CSL, CMC1, FYM and CMC2 explained 6%, 4%, 3%, 4% and 1% of the total variance respectively. The first two axes of the PCA (λ= 0.26 and 0.12 respectively) were used to construct the PCA ordination diagram (Fig. 1). The amount of variance in species data explained by the first two axes was 35% and 16% respectively. Only the vectors of these species that had a fit of 4% or more to the diagram and occurred in at least 5 plots were depicted in the ordination diagram. The positive side of the first ordination axis is related to fertilisation system CMC1 with an inter-set correlation coefficient of 0.35**. Species characterising the weed seed bank of CMC1 plots were *P. annua*, *Matricaria chamomilla* L. and *Sonchus oleraceus* L.. Species such as *S. nigrum*, *C. hirsutum, G. quadriradiata,* were ordinated towards the negative side of the first ordination axis and were related to fertilisation system VFG, with an inter-set correlation coefficient of -0.22*. The positive side of the second ordination axis is related to fertilisation system MIN N (inter-set correlation coefficient of 0.50**) and is characterised by *C. album, C. polyspermum, L. purpureum* and *P. maculosa.* The negative side of the second ordination axis is related to

295 fertilisation systems FYM (inter-set correlation coefficient of -0.23**) and CSL (inter-set
296 correlation coefficient of -0.24**). Species associated with these fertilisation systems were
297 *Epilobium ciliatum* Rafin. and to a lesser extend *G. uliginosum* and *P. major* subsp*. major*.
298
299 *Fig. 1 near here*
300
301 *Crop DM yield*
302 The DM yields of cabbage heads and leaves, beet roots and leaves, ryegrass and maize
303 were similar for all fertilised plots, except for CSL plots showing lower DM yields of beet roots
304 and maize and for MIN N plots showing a lower yield of ryegrass (Table 4). Hence, the
305 applied amounts of nutrients through the amendments and fertilisers were correctly
306 calculated.
307
308 *Table 4 near here*
309
310 *Weed seed bank density in relation to soil organic C content, pH-KCl and microbial biomass*
311 Soil organic C content in minerally fertilised plots was significantly lower than in organically
312 amended plots (Table 5). Within organically amended plots, no significant differences in soil
313 organic carbon content were found. FYM, VFG and CSL plots showed significantly higher
314 pH-KCl than CMC1 and MIN N plots. Fertiliser type affected microbial biomass (indirectly
315 measured by PLFA content) and community composition (Table 5). All organically fertilised
316 plots showed significantly higher PLFA contents of actinomycetes, Gram-positive and Gram-
317 negative bacteria than minerally amended plots. Within compost amended plots (VFG,
318 CMC1, CMC2), no significant differences in total microbial, fungal and bacterial PLFA
319 contents were found. Within organically fertilised plots, CMC1 plots had higher fungal PLFA
320 content and lower bacteria to fungi ratio than FYM and CSL plots.
321
322 *Table 5 near here*

323

324 At plot level, soil organic carbon content is significantly ($P < 0.05$) positively related to
325 microbial biomass with a linear correlation coefficient of 0.48. Total weed seed bank density
326 was significantly negatively correlated with soil organic carbon content (r= -0.44), total
327 microbial biomass (r = -0.34) and AFLP content of actinomycetes (Table 6, Figure 2).

328

329 *Fig. 2 near here*

330

331 Seed bank densities of the highly competitive weed *C. polyspermum* were
332 significantly negatively correlated with bacterial (actinomycetes, Gram-positive and Gram-
333 negative bacteria) and total microbial biomass, and pH-KCl, but not with soil organic C
334 content (Table 6). Unlike *C. polyspermum*, seed bank density of *P. aviculare* was significantly
335 positively correlated with bacterial and total microbial biomass. Seed density of *P. major*
336 subsp. *major* revealed a weak negative correlation with biomass of Gram-positive bacteria.
337 Seed densities of *C. bursa-pastoris*, *P. major* subsp. *major, P. maculosa* and *S. nigrum* were
338 not significantly correlated with total microbial, fungal or bacterial (except for the correlation
339 between density of *P. major* subsp. *major* and AFLP content of Gram-positive bacteria)
340 biomass despite their significant negative correlations with soil organic C content.

341

342 Table 6 *near here*

343

344 **Discussion**

345

346 No data on initial weed seed bank size are available, but the field was uniformly cropped with
347 maize before 2005, pesticidal control was uniform across the experimental site and weed
348 infestations were moderate. Seed rain from outside the plots is assumed to be very low and
349 identical across all plots because wind dispersible seeds were hardly produced in the maize
350 monoculture fields and intensively mown boundaries bordering the experimental field. Thus,

351 it is reasonable to attribute differences in weed seed bank densities mainly to the treatments
352 imposed after 2005.

353 All organically amended soils revealed similar soil organic carbon contents, except for
354 the slurry amended soils showing lower values. The lower soil organic carbon content in
355 slurry amended plots resulted in a lower amount of microbial biomass compared with plots
356 receiving more stabile carbon forms. At plot level, soil carbon content was significantly
357 correlated with microbial biomass.

358 Total weed seed bank density was lowest in compost amended plots (CMC1, CMC2
359 and VFG) and highest in slurry amended plots. These differences are unlikely to be
360 explained by differences in crop competitiveness or amounts of viable seeds in the organic
361 fertilisers. Indeed, aboveground DM biomass production was similar for all fertilised plots
362 and content of germinable seeds in the applied organic fertilisers was very low compared to
363 the seed bank content. Unlike manure, compost is not a significant source of viable seeds if
364 properly composted (Eghball & Lesoing, 2000). Manure or slurry may only be a relatively
365 major source of weed seeds, if soil seed bank numbers are low (Pleasant & Schlather,
366 1994). The higher seed bank numbers in slurry amended plots cannot be attributed to a lack
367 of dormancy breaking: although these plots mostly did not receive extra ammonium nitrate (a
368 well-known dormancy breaking agent), applied cattle slurry itself contained large amounts of
369 mineral N. Therefore, it is more reasonable to attribute differences in seed bank density to
370 differences in seed decay, seed production or seed predation.

371 Compost amended plots (CMC1, CMC2 and VFG) showed higher total microbial,
372 fungal and bacterial (except for Gram-negative bacteria) biomass and lower bacteria to fungi
373 ratios. Indeed, recalcitrant compounds are mainly decomposed by fungi, whereas readily
374 decomposable compounds, such as organic acids and carbohydrates present in manure and
375 slurry, are preferentially utilised by soil bacteria (Marschner *et al*., 2003).

376 Definite evidence that soil microorganisms were responsible for the lower seed bank
377 density in compost plots cannot be provided, because differences in total microbial, fungal
378 and bacterial biomass were not significant in the short term. Nevertheless, lower seed bank

densities were found in plots with high microbial activity, indicating that microbial seed deterioration might be higher in these plots, since nor specific low seed production, nor seed predation are assumed to be responsible for the low seed bank densities. Seed production was assumed to be low particularly for summer-germinating species because of the residual effect of soil herbicides, high crop competitiveness and year-round soil coverage. Seed predation is usually low in agricultural systems with intensive soil disturbance, seed burial by tillage and lack of habitats for predators and for species with hard seed pericarps (Brust & House, 1988).

Total weed seed density in minerally fertilised plots was comparable to the weed density in compost amended plots despite their lower soil organic carbon content, total microbial, fungal and bacterial biomass. The well known stimulating effect of ammonium nitrate on seed germination of many species (Karssen & Hilhorst, 1992), combined with mortality due to spring herbicide application offers an acceptable explanation.

Plots amended with more stable carbon compounds, in particular VFG and CMC2 plots, showed lower seed densities of *L. purpureum*, *C. album*, *C. bursa-pastoris, P. maculosa, P. major* subsp. *major, P. annua* and *S. nigrum* than plots amended with more readily decomposable compounds (CSL) or synthetic fertiliser (MIN N). These findings are in line with studies reporting lower weed infestations by *C. bursa-pastoris* (Fennimore & Jackson, 2003) and *C. album* (Gallandt *et al.*, 1999) in soils amended with organic fertilisers. Within microbial groups, weed seed bank numbers on plot level were best correlated with biomass of Gram-positive bacteria. Observed significant correlations between biomass of Gram-positive bacteria and seed bank densities were negative for *C. polyspermum* and *P. major* subsp. *major,* both species with long-term persistent hard-coated seeds, but correlations were positive for *S. vulgaris* and *S. media* both species with transient or short-term persistent seed banks. Interspecific differences in resistance against microbial breakdown of seeds may be responsible for this differential response. Indeed, weed species with short-lived seed banks appear to invest more in chemical defense than species with highly persistent seed banks that rely mainly on physical seed protection (Davis *et al.*, 2008).

Hence, species with long-term persistent seed banks are more vulnerable to management actions that reduce physical integrity of the weed seed coat, such as the use of organic fertilisers that stimulate microbial activity. It is reasonable to explain the observed negative correlation between seed bank densities of *C. polyspermum* and *P. major* subsp. *major* and bacterial and total microbial biomass at plot level by the combined action of enhanced microbial breakdown of their hard seed coat and weak chemical defense properties of their seed coat. Apart from seed mortality by microbial invasion and decomposition of seeds, some microorganisms are known to soften the impermeable seed coat by enzymes, thus enabling seed germination (Gogue & Emino, 1979). Unlike former hard-coated species, *P. aviculare* was positively correlated with bacterial and total microbial biomass, despite its hard seediness. It is well known that all plant parts of *P. aviculare* contain phytochemical constituents, such as tannins, saponins and flavonoids with broad spectrum activity against bacteria. So, diffusion of antimicrobial substance from *P. aviculare* seeds might limit or inhibit potential seed decomposers particularly in bacteria-rich soils, adding a good chemical defense strategy to a good physical defense strategy.

Fertiliser form and quality influenced weed seed bank composition, as shown by multivariate seed bank analysis. The seed bank of minerally fertilised plots was characterised by species with hard seed coats, such as *C. polyspermum, P. maculosa* and *C. album*. Probably, seeds of these species were less prone to microbial deterioration under prevailing conditions of low microbial activity. Plots amended with VFG compost were associated with late germinating weeds preferring nutrient-rich organic soils, such as *G. quadriradiata* and *C. hirsuta* (winter annual).

Conclusions

The results presented in this study showed evidence for a significant short term effect of the type and quality of organic amendments on the weed seed bank: seed bank numbers were higher in plots amended with cattle slurry than in plots amended with compost with low C:N ratio. Differences in seed bank numbers between compost and manure were moderate but

might become more pronounced in the long term. Hence, fertiliser management can be a promising and sustainable tool in integrated weed control strategies aiming at depleting the soil seed bank. The correlation study provided indirect evidence that increased organic matter content or microbial biomass (or both) have a potential to affect soil seed banks, particularly those with high abundance of long-term persistent species with hard-coated seeds. However, more fundamental research is necessary to provide conclusive evidence.

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Figure legends

Fig. 1 PCA ordination plot of weed seed bank species (depicted with BAYER codes) and environmental variables. CAPBP, *Capsella bursa-pastoris*; CARHI, *Cardamine hirsuta*; CERGL, *Cerastium glomeratum*; CHEAL, *Chenopodium album*; CHEPO, *Chenopodium polyspermum*; EPIAC, *Epilobium ciliatum*; GASCI, *Galinsoga quadriradiata*; GNAUL, *Gnaphalium uliginosum*; IUNBU, *Juncus bufonius*; LAMPU, *Lamium purpureum*; MATCH, *Matricaria chamomilla*; PLAMA, *Plantago major* subsp*. major;* POAAN, *Poa annua*; POLAV, *Polygonum aviculare*; POLPE, *Polygonum maculosa*; SAIPR, *Sagina procumbens*; SENVU, *Senecio vulgaris*; SOLNI, *Solanum nigrum*; SONOL, *Sonchus oleraceus*; STEME, *Stellaria media; TAROF, Taraxacum officinale.* Solid dots represent centroids of six fertilization systems: FYM, farmyard manure; VFG, vegetable fruit and garden compost; CMC1, farm compost with high C:N ratio 20-40; CMC2, farm compost with low C:N ratio; CSL, cattle slurry; MIN N, only mineral N.

Fig. 2 Linear regression between total weed seed bank density and total microbial PLFA content (left) and soil organic carbon content (right).

TABLES

538 **Table 1** Applied amounts of organic amendments and their C and N content, and the applied
539 amount of extra mineral N for the fertilizer systems (FYM, farmyard manure; VFG, vegetable
540 fruit and garden compost; CMC1, farm compost with high C:N ratio 20-40; CMC2, farm
541 compost with low C:N ratio; CSL, cattle slurry; MIN N, only mineral N)

Fertilization system	Organic fertilizer C content (g kg^{-1} fresh matter)	N content (g kg^{-1} fresh matter)	Applied amount (kg ha^{-1})	Applied extra mineral N (kg ha^{-1})
Application 1 (21.04.2005, 4000 kg C ha^{-1}): prior to sowing of fodder beet				
FYM	62.2	4.7	64329	105
VFG	179.4	15.2	22303	114
CMC1	71.4	1.7	56007	165
CMC2	59.7	2.8	67058	165
CSL + straw	26.7[3] / 378.0	3.9[3] / 5.5	77382[2]+ 4704	
MIN N	-	-	-	165
Application 2 (06.10.2005, 4000 kg C ha^{-1}): prior to sowing of winter wheat				
FYM	106.8	6.8	67453	81 + 97[1]
VFG	175.7	14.5	22770	88 + 98[1]
CMC1	71.8	3.1	55718	91 + 99[1]
CMC2	77.9	7.2	51348	89 + 97[1]
CSL + beet leaves	20.4[3] / 49.1	2.8[3] / 3.4	74698[2] + 53636	74 + 94[1]
MIN N	-	-	-	91 + 98[1]
Application 3 (07.09.2006, 1500 kg C ha^{-1}): prior to sowing of phacelia				
FYM	104.2	6.7	14398	66
VFG	183.2	15.5	8188	67
CMC1	77.6	4.1	19330	86
CMC2	63.1	3.4	23757	86
CSL	26.4[3]	3.8[3]	56754[2]	
MIN N	-	-	-	86
Application 4 (02.05.07, 2000 kg C ha^{-1}): prior to planting of red cabbage				
FYM	104.6	6.1	19125	106
VFG	139.6	9.1	14329	103
CMC1	192.0	3.6	10417	170
CMC2	91.0	6.0	21986	162
CSL	28.1[3]	3.2[3]	71287[2]	
MIN N	-	-	-	162
Application 5 (21.05.2008, 1101 kg C ha^{-1}): prior to sowing of perennial ryegrass				
FYM	76.0	5.9	14488	66
VFG	130.6	9.0	8436	50
CMC1	125.2	4.7	8800	97
CMC2	130.3	9.3	8452	125
CSL	34.2	4.5	32222	
MIN N	-	-	-	109
Application 6 (11.05.2009, 3259 kg C ha^{-1}): prior to sowing of fodder maize				
FYM	74.3	2.8	43889	190
VFG	130.6	9.0	24960	164
CMC1	124.4	4.7	26198	235
CMC2	84.7	9.3	38474	248
CSL	34.7	3.9	93872	
MIN N	-	-	-	232

[1] Fractionated N dose applied on 23.03.2006 and 26.04.2006

[2] L ha^{-1}

542 [3] g L^{-1}

543 **Table 2** Sowing date, harvest date and pesticide applications in subsequent crops from 2005

544 to 2009

Year	Crop	Sowing date	Harvest date	Pesticide application†		
				Dose	Type	Date
2005	fodder beet	22.04	04.10	3 kg ha^{-1} Goltix + 0.6 L ha^{-1} Vegelux	herbicide	24.05
				3 kg ha^{-1} Goltix + 0.6 L ha^{-1} Vegelux + 1 L ha^{-1} Eloge	herbicide	02.06
2006	winter wheat	07.10.2005	07.08	3 L ha^{-1} Azur	herbicide	07.04
				1 L ha^{-1} Horizon	fungicide	03.05
	phacelia	07.09		1 L ha^{-1} Eloge	herbicide	16.10
2007	red cabbage	22.05	02.10	Dursban, 100ml per plant (0.15% solution)	insecticide	22.05
				4 L ha^{-1} Ramrod	herbicide	30.05
				1.65 kg ha^{-1} Lentagran	herbicide	10.06
				1.5 L ha^{-1} Okapi	insecticide	18.06, 27.06 and 12.07
2008	perennial ryegrass	21.05	02.07, 01.08 and 12.11			
2009	silage maize	11.05	17.09	0.9 L ha^{-1} Frontier + 0.9 L ha^{-1} Mikado + 0.9 L ha^{-1} Samson 4 SC	herbicide	05.06

† Frontier (900 g L^{-1} dimethenamid, EC, BASF); Goltix (70% metamitron, WG, MAKTESHIM-AGAN); Mikado (300 g L^{-1} sulcotrione, SC, BAYER); Samson 4 SC (40 g L^{-1} nicosulfuron, SC, BELCHIM); Vegelux (832 g L^{-1} liquid paraffin, EC, SAFIC-ALCAN); Eloge (108 g L^{-1} haloxyfop-R-methyl, EC, DOW AGRO); Azur (20 g L^{-1} diflufenican + 100 g L^{-1} ioxynil + 400 g L^{-1} isoproturon, SC, BAYER); Horizon (250 g L^{-1} tebuconazole, EW, BAYER); Dursban (480 g L^{-1} chlorpyrifos, EC, DOW AGRO); Ramrod (480 g L^{-1} propachlor, SC, MONSANTO); Lentagran (45% pyridate, WP, BELCHIM); Okapi (5 g L^{-1} lambda-cyhalothrin + 100 g L^{-1} pirimicarb, EC,

545 SYNGENTA).

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547

548 **Table 3** Mean seed bank density (seedlings m^{-2}) for the main weed species emerged from
549 the seed bank for all fertilization systems (FYM, farmyard manure; VFG, vegetable fruit and
550 garden compost; CMC1, farm compost with high C:N ratio 20-40; CMC2, farm compost with
551 low C:N ratio; CSL, cattle slurry; MIN N, only mineral N). Values are means ± standard errors
552

	FYM	VFG	CMC1	CMC2	CSL	MIN N
Species						
Capsella bursa-pastoris	895 ± 128.4 ^{ab}	721 ± 264.7 ^{ab}	1078 ± 191.7 ^{ab}	555 ± 200.0 ^{b}	1434 ± 528.2 ^{a}	904 ± 181.3 ^{ab}
Cardamine hirsuta	17 ± 16.6 ^{a}	41 ± 41.4 ^{a}	8 ± 8.3 ^{a}	41 ± 15.9 ^{a}	25 ± 24.9 ^{a}	17 ± 16.6 ^{a}
Cerastium glomeratum	99 ± 52.4 ^{ab}	99 ± 44.9 ^{ab}	41 ± 31.4 ^{b}	75 ± 8.3 ^{ab}	108 ± 34.2 ^{a}	41 ± 15.9 ^{ab}
Chenopodium album †	124 ± 24.9 ^{ab}	116 ± 55.0 ^{ab}	257 ± 68.2 ^{a}	108 ± 59.6 ^{b}	356 ± 247.5 ^{a}	323 ± 122.9 ^{a}
Chenopodium polyspermum †	754 ± 526.9 ^{ab}	688 ± 507.6 ^{ab}	274 ± 108.6 ^{b}	356 ± 174.1 ^{ab}	522 ± 208.6 ^{ab}	1020 ± 386.3 ^{a}
Gnaphalium uliginosum	191 ± 84.9 ^{a}	116 ± 58.2 ^{a}	58 ± 36.8 ^{a}	66 ± 35.8 ^{a}	116 ± 28.7 ^{a}	99 ± 77.8 ^{a}
Lamium purpureum	75 ± 53.1 ^{ab}	8 ± 8.3 ^{b}	91 ± 36.8 ^{a}	41 ± 15.9 ^{ab}	116 ± 82.9 ^{a}	50 ± 9.6 ^{ab}
Plantago major subsp. *major* †	986 ± 456.3 ^{ab}	713 ± 365.6 ^{ab}	356 ± 147.9 ^{b}	414 ± 193.6 ^{b}	1550 ± 565.7 ^{a}	903 ± 583.2 ^{ab}
Poa annua	812 ± 405.3 ^{a}	274 ± 56.4 ^{a}	1442 ± 707.6 ^{a}	348 ± 113.7 ^{a}	2926 ± 2382.2 ^{a}	738 ± 549.1 ^{a}
Polygonum aviculare †	199 ± 77.8 ^{a}	91 ± 36.8 ^{ab}	191 ± 31.4 ^{a}	133 ± 70.3 ^{ab}	66.3 ± 30.3 ^{b}	66 ± 23.4 ^{b}
Polygonum maculosa †	33 ± 23.4 ^{a}	91 ± 53.1 ^{a}	66 ± 23.4 ^{a}	66 ± 35.8 ^{a}	108 ± 65.4 ^{a}	124 ± 15.9 ^{a}
Senecio vulgaris	75 ± 43.6 ^{a}	50 ± 28.7 ^{a}	75 ± 15.9 ^{a}	58 ± 8.3 ^{a}	157 ± 66.8 ^{a}	25 ± 15.9 ^{a}
Solanum nigrum †	91 ± 31.4 ^{a}	124 ± 15.9 ^{a}	108 ± 8.3 ^{a}	108 ± 34.2 ^{a}	191 ± 90.2 ^{a}	133 ± 30.3 ^{a}
Stellaria media	182 ± 84.0 ^{ab}	158 ± 68.2 ^{b}	182 ± 28.7 ^{ab}	232 ± 64.9 ^{ab}	580 ± 198.3 ^{a}	141 ± 62.6 ^{b}
Total seed bank	4783 ± 940.2 ^{ab}	3382 ± 977.3 ^{b}	4543 ± 834.8 ^{ab}	2710 ± 424.3 ^{b}	8463 ± 3216.4 ^{a}	4741 ± 956.7 ^{ab}

† Species with hard seed coat.

No significant differences between figures with the same letter (Fisher's LSD on fourth-root transformed data, P = 0.05), comparison
553 within rows only.

554

555 **Table 4** DM yield (t ha^{-1}) of subsequent crops for all fertilization systems (FYM, farmyard
556 manure; VFG, vegetable fruit and garden compost; CMC1, farm compost with high C:N ratio
557 20-40; CMC2, farm compost with low C:N ratio; CSL, cattle slurry; MIN N, only mineral N).
558 Values are means ± standard errors

Year	Crop	Crop part	Fertilization system					
			FYM	VFG	CMC1	CMC2	CSL	MIN N
2005	beet	roots	16.5 ± 0.71	16.0 ± 0.34	16.8 ± 0.81	17.7 ± 0.26	13.3 ± 1.14	18.4 ± 0.80
		leaves	6.9 ± 0.17	6.8 ± 0.42	7.3 ± 0.38	7.5 ± 0.54	5.4 ± 0.28	7.1 ± 0.26
2007	cabbage	heads	7.5 ± 0.24	7.5 ± 0.06	7.1 ± 0.37	7.4 ± 0.30	7.5 ± 0.45	7.1 ± 0.15
		leaves	6.7 ± 0.17	6.8 ± 0.19	6.2 ± 0.32	6.7 ± 0.07	7.0 ± 0.39	6.7 ± 0.53
2008	ryegrass	aboveground biomass	6.1 ± 0.22	5.2 ± 0.28	5.6 ± 0.23	6.7 ± 0.10	5.1 ± 0.33	4.6 ± 0.49
2009	maize	aboveground biomass	22.3 ± 0.39	20.0 ± 0.54	21.0 ± 0.65	21.7 ± 0.38	17.8 ± 0.64	20.3 ± 0.44

559 Confidence intervals of the estimates may be calculated by multiplying the standard error by $t_{0.975} = 1.96$

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562 **Table 5** Total amount of PLFAs, amount of PLFAs of fungi and bacteria, bacteria to fungi
563 ratio and soil organic carbon content for all fertilization systems (FYM, farmyard manure;
564 VFG, vegetable fruit and garden compost; CMC1, farm compost with high C:N ratio 20-40;
565 CMC2, farm compost with low C:N ratio; CSL, cattle slurry; MIN N, only mineral N). Values
566 are means ± standard errors

	FYM	VFG	CMC1	CMC2	CSL	MIN N
Amount of PLFAs : (ng g^{-1} soil)						
Total	7638 ± 422.5 ^{a}	7362 ± 363.5 ^{ab}	7456 ± 313.0 ^{ab}	7415 ± 160.5 ^{ab}	6936 ± 297.0 ^{b}	5591 ± 162.5 ^{b}
Fungi	276 ± 14.9 ^{b}	323 ± 23.7 ^{ab}	382 ± 35.9 ^{a}	306 ± 9.9 ^{ab}	274 ± 19.1 ^{b}	275 ± 41.7 ^{b}
Actinomycetes	396 ± 15.7 ^{a}	377 ± 20.1 ^{a}	395 ± 14.7 ^{a}	389 ± 6.8 ^{a}	366 ± 20.4 ^{a}	306 ± 6.1 ^{b}
Gram-positive	1255 ± 106.6 ^{a}	1222 ± 102 ^{a}	1217 ± 46.8 ^{a}	1245 ± 66.4 ^{a}	1188 ± 17.1 ^{a}	841 ± 60.6 ^{b}
Gram-negative	154 ± 9.0 ^{a}	143 ± 11.1 ^{ab}	141 ± 4.2 ^{ab}	137 ± 0.5 ^{b}	143 ± 2 ^{ab}	116 ± 5.9 ^{c}
Bacteria/fungi ratio	6.7 ± 0.11 ^{a}	5.6 ± 0.42 ^{ab}	4.8 ± 0.32 ^{b}	5.9 ± 0.14 ^{ab}	6.5 ± 0.44 ^{a}	5.1 ± 0.87 ^{b}
Soil organic C (%)	1.26 ± 0.056 ^{a}	1.27 ± 0.074 ^{a}	1.21 ± 0.052 ^{a}	1.26 ± 0.034 ^{a}	1.18 ± 0.071 ^{ab}	1.12 ± 0.044 ^{b}
pH-KCl	6.0 ± 0.06 ^{ab}	6.1 ± 0.06 ^{a}	5.8 ± 0.03 ^{c}	5.9 ± 0.08 ^{b}	6.0 ± 0.04 ^{ab}	5.6 ± 0.11 ^{d}

No significant differences between figures with the same letter (Fischer's LSD , P = 0.05), comparison
567 within rows only.

568 **Table 6** Pearson's correlation coefficients between fourth-root transformed weed seed bank
569 densities of main seed bank species and bacterial, fungal and total microbial PLFA content
570 and soil organic carbon content
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	Total	Fungi	Gram-positive	Gram-negative	Actino-mycetes	Soil organic C content	pH-KCl
Species:							
Capsella bursa-pastoris	-0.19	0.03	-0.16	0.03	-0.21	-0.39 *	-0.19
Cardamine hirsuta	0.19	-0.10	0.23	0.14	0.12	-0.06	0.04
Cerastium glomeratum	0.07	-0.35 *	0.26	0.18	-0.03	0.09	0.34 *
Chenopodium album	-0.25	-0.01	-0.10	-0.04	-0.28	-0.23	-0.11
Chenopodium polyspermum	-0.57 **	-0.23	-0.63 ***	-0.57 **	-0.52 **	-0.03	-0.37 *
Gnaphalium uliginosum	0.03	-0.02	0.04	-0.05	-0.01	0.03	0.31
Lamium purpureum	-0.17	-0.05	-0.01	-0.03	-0.12	-0.12	-0.23
Plantago major subsp. *major*	-0.25	-0.21	-0.30 *	-0.17	-0.26	-0.29 *	0.00
Poa annua	-0.04	-0.28	0.13	0.16	-0.11	-0.15	0.06
Polygonum aviculare	0.36 *	0.00	0.43 *	0.40 *	0.34 *	0.12	0.25
Polygonum maculosa	-0.01	0.16	0.09	0.06	-0.18	-0.59 **	-0.25
Senecio vulgaris	0.19	-0.09	0.33 *	0.27	0.18	0.21	0.14
Solanum nigrum	-0.09	-0.03	-0.06	-0.14	-0.20	-0.40 *	-0.23
Stellaria media	0.14	-0.12	0.34 *	0.28	0.18	0.17	0.43 *
Total seed bank	-0.34 *	-0.20	-0.23	-0.12	-0.41 *	-0.44 *	-0.10

572 * P < 0.05; ** P < 0.01; P < 0.001 otherwise P > 0.05.
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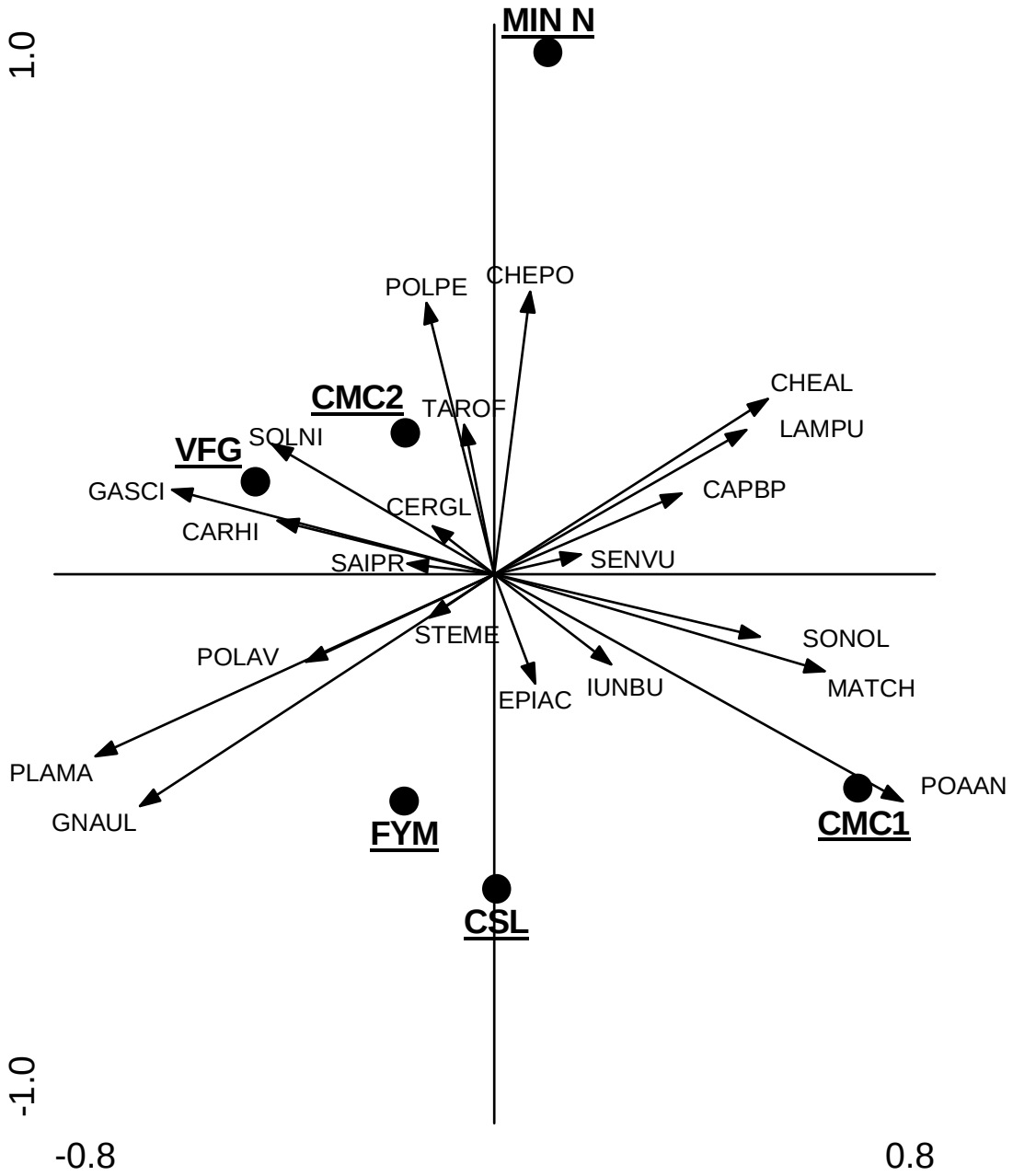

575

576 **Fig. 1** PCA ordination plot of weed seed bank species (depicted with BAYER codes) and
577 environmental variables. CAPBP, *Capsella bursa-pastoris*; CARHI, *Cardamine hirsuta*;
578 CERGL, *Cerastium glomeratum*; CHEAL, *Chenopodium album*; CHEPO, *Chenopodium*
579 *polyspermum*; EPIAC, *Epilobium ciliatum*; GASCI, *Galinsoga quadriradiata*; GNAUL,
580 *Gnaphalium uliginosum*; IUNBU, *Juncus bufonius*; LAMPU, *Lamium purpureum*; MATCH,
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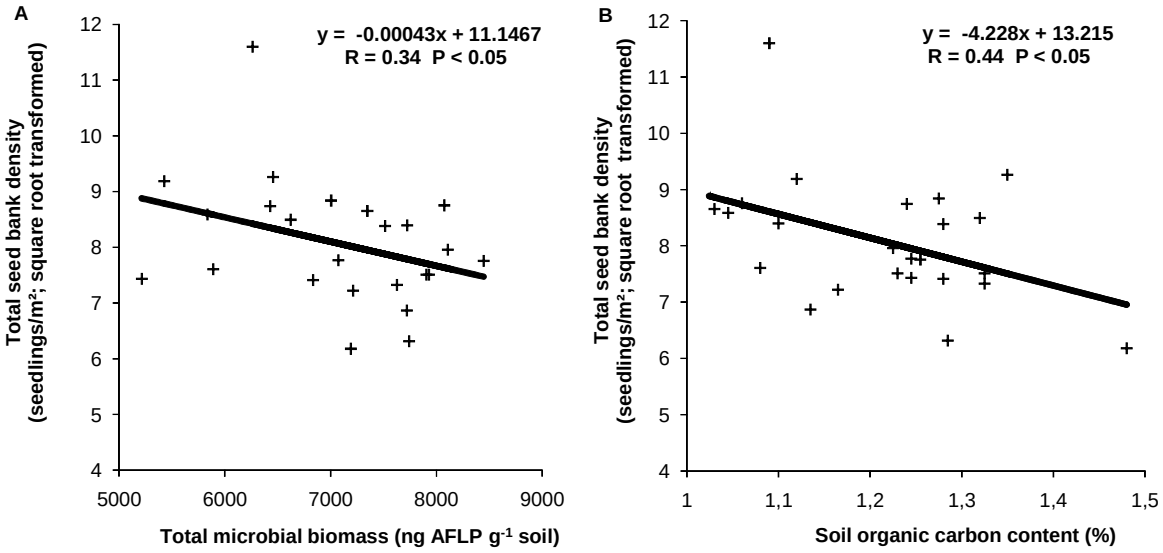
584 *media; TAROF, Taraxacum officinale.* Solid dots represent centroids of six fertilization
585 systems: FYM, farmyard manure; VFG, vegetable fruit and garden compost; CMC1, farm
586 compost with high C:N ratio 20-40; CMC2, farm compost with low C:N ratio; CSL, cattle
587 slurry; MIN N, only mineral N.



588

589 **Fig. 2** Linear regression between total weed seed bank density and total microbial PLFA

590 content (left) and soil organic carbon content (right).

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