Decomposition of the biodiesel by-product, crude glycerol, in soil

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Abstract

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The disposal of crude glycerol, the biodiesel by-product, may become an economic or environmental problem in cases where no trading of this material is possible. This study aimed at evaluating the decomposability in soil of the unpurified glycerol fraction taken after the transesterification of oil using sodium hydroxide as a catalyst. The immediate effect of glycerol incorporation was a considerable increase in soil pH. In soil samples characterized by low biological activity this pH increase did not permit microbial development although the time lag before the growth of microorganisms feeding on glycerol was shortened after the addition of a nitrogen inorganic source. On the contrary, in soils with higher organic matter content and active microbial communities, excess alkalinity was rapidly eliminated and glycerol C mineralization progressed with a relatively high rate reaching 53% of initial C added after 2.5 months of incubation when an inorganic nitrogen source was available. It is concluded that results allow for further consideration of the possibility of spreading glycerol on soil or including it in compost piles.

Keywords: glycerine; C mineralization; soil respiration

Biofuels, such as bioethanol and biodiesel, represent attractive energy sources because they are made from renewable materials. Moreover, they could partly substitute fossil fuel consumption, offset CO_2 emissions and contribute to mitigation of climate change. The use of biofuels will probably continue to grow, as the availability of petroleum is limited (Hill et al. 2006). Biodiesel production in particular increased worldwide from 2.2 Mt/year in 2002 to 12.9 Mt/year in 2009 (IEA 2009).

Although there are three routes to biodiesel production from vegetable oils and animal fats almost all biodiesel is produced using base catalyzed transesterification as it is the most economical process requiring only low temperatures and pressures and producing a 98% conversion yield (Ma, Hanna 1999). During the transesterification process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkali. The alcohol reacts with the fatty acids to form the mono-alkyl ester, or biodiesel and crude glycerol. In most production methanol or ethanol is the alcohol used and is base catalyzed by either potassium or sodium hydroxide (Fukuda et al. 2001).

The main by-product of this procedure is the glycerol fraction. From 1 kg of oil approximately 200 g of this fraction is produced (Meher et al. 2006). Although pure glycerol, a colourless, odourless, viscous, nontoxic liquid with a very sweet taste is one of the most versatile and valuable chemical substances, the biodiesel by-product named as glycerol is a mixture containing different amounts of soap, alcohol, catalyst, and water and usually cannot be used before a purification process (Gerpen 2005). Methanol for example, is one of the reactants in the production of biodiesel from fats and

oils. Excess methanol is typically used to drive the transesterification reaction forward, and the unreacted methanol finally ends up in the glycerol fraction due to its polar nature. Glycerol was used in the pharmaceutical industry, as fuel or as animal feed (Cerrate et al. 2006). There are factors restricting these uses such as the purity of glycerol, which can have negative impacts on animal health.

Separating pure glycerol is not a very cheap procedure. In cases where its exploitation is non-profitable, the disposal of this fraction may create problems to the viability of biodiesel enterprises. This is particularly true in countries with non-developed markets of glycerol.

There is currently lack of information available in the literature on the eventual risks or benefits arising from the disposal of crude glycerol on the soil surface. If shown to be easily decomposed by soil microbes, it could be used as a C source for composting organic materials or even as a soil amendment. The stimulation of microbial activity in soil or compost piles was often attributed to increased inputs of C-rich organic materials and nutrients (Watts et al. 2010). It could, however, pose environmental risks if it decomposes slowly and is ultimately accumulated in soil.

The present work is an attempt to determine the decomposability of biodiesel production by-product of glycerol in soil incubated under controlled environment conditions.

MATERIAL AND METHODS

The experiment consisted of three laboratory incubations, which were carried out successively so that observational evidence of the initial ones was to be taken into account in the design of the following. Soil samples were mixed with crude glycerol in plastic containers and incubated under controlled temperature and moisture conditions.

The crude glycerol that was used was obtained from the procedure of biodiesel production of a pilot unit (Fuel Pod Biodiesel Processor, Green Fuels, Cheltenham, UK) possessed by the Agricultural Research Institute of Cyprus. Feedstock material was composed by two thirds of commercial seed oil and one third of frying oil. The catalyst selected for the production of the material used in the experiment was sodium hydroxide. No distillation or other purification procedure followed the separation of the crude glycerol phase from biodiesel. The term "glycerol" will be referred from now on to this crude glycerol fraction of biodiesel production. This material was characterized by a specific weight of 1.09 g/ml, contained 22% pure glycerol whereas 40% v/v was moisture and the catalyst remnant. Carbon content was 25%.

Soils were air dried and sieved with a 2 mm sieve. Visible plant residues and roots were removed by hand. Soil samples of 75 g contained in plastic pots were remoistened by adding water corresponding to 80% of the water holding capacity (WHC) of soils. In treatments involving addition of NH_4NO_3 or KNO_3, salt was diluted to the corresponding amount of water used to remoisten soil samples. Glycerol was spread by a pipette on the surface of soil samples and left overnight to be fully absorbed by soil before starting incubation. Incubations were carried out in 2-l gas-tight jars at 20°C. To maintain a vapour saturated atmosphere inside the jars water was always kept at their bottom. Evolved CO_2 was captured in a vial containing 40 ml of 0.5 mol/l NaOH and the quantity of CO_2 absorbed in the alkali was determined by titration with 0.2 mol/l HCl (Berg, Laskowski 2006).

To test whether the initial condition of the soil microbial community was a considerable variable for the outbreak and the overall dynamics of glycerol decomposition, the soils used were characterized by presumably different degrees of biological activity. First incubation was carried out using soil

Table 1. Selected properties of the soils used in the study

	Soil texture	NH_4^+ (ppm)	NO_3^- (ppm)	Total N (%)	Organic C (%)	EC (mS/cm)	$CaCO_3$ (%)
Soil 1	sandy clay loam	3.0	3.9	0.022	0.30	0.096	3.7
Soil 2	sandy clay loam	6.2	5.0	0.085	0.93	0.143	15

EC– electrical conductivity; Soil 1 – used in 1st incubation; Soil 2 – used in 2nd and 3rd incubation. Soil sample 1 was taken from a soil pile, which remained without any plant development for two years. The pile came from the 20–40 cm soil layer of an abandoned agricultural field. Soil sample 2 was a topsoil sampled from a wheat field in November (2nd incubation) or April (3rd incubation)

samples taken from a soil pile, which remained without any plant development for two years (Table 1). This soil originated from an abandoned agricultural field from which topsoil layer (0–20 cm) had been removed. Samples were incubated either (a) with no organic addition (control treatment) or mixed with (b) 5ml of glycerol (glycerol-5 treatment), (c) 10 ml of glycerol (glycerol-10 treatment), (d) 1.1 g NH_4NO_3 only and (e) 1.1 g NH_4NO_3 + 5 ml glycerol. NH_4NO_3 was used as an inorganic source of nitrogen for microbial development. Carbon-to-nitrogen ratio of the overall input in treatment (e) was 3.54.

Second incubation was carried out with soil coming from a wheat field (Table 1). It was sampled at the end of autumn before seeding and before first rains of the year. Treatments there were: (a) 75 g of soil only (control treatment), (b) soil mixed with 5 ml of glycerol, (c) soil mixed with 5 ml glycerol and a soil inoculum, (d) soil with 5 ml glycerol and 1.1 g NH_4NO_3 and (e) soil with glycerol + NH_4NO_3 + inoculum. Inoculum consisted of small soil surface parts containing fungi hyphae. This soil inoculum was taken from a pre-treatment during which glycerol was spread on the soil surface together with NH_4NO_3 and left outdoors for four weeks.

Third incubation was carried out using soil of the same wheat field as previously, which, however, was sampled in April towards the end of the vegetative period of wheat when soil activity was presumably greater than in autumn. Treatments there were: (a) soil only (control treatment), (b) soil mixed with 5ml of glycerol, (c) soil mixed with 5 ml glycerol and 1.1 g NH_4NO_3, (d) soil with 5 ml glycerol and 1.39 g KNO_3. C-to-N ratio of the overall input in this final treatment was 7.04. The addition of NH_4NO_3 was accompanied by a reduction of pH (during addition an ammonia smell was evident). It would not be possible, therefore, to distinguish whether eventually greater decomposition rates would arise from the presence of an N source or from the more favourable for microbial activity pH conditions. KNO_3 was added (treatment (d)) to provide nitrogen to microorganisms without affecting soil pH. The quantity of N added (0.19 g N) was half that of NH_4NO_3 (0.385 g N), assuming that most quantity of ammonium ions would be released as NH_3.

At all three incubations, three jars containing alkali trap but no soil were used as blank. Soil samples were prepared in double and one of them was used for the measurement of the initial $pH_{(H_2O)}$ (1:2.5 soil to water ratio). After incubation the pH of samples was measured again. Incubations and pH measurements were carried out in three replications at each treatment.

Analysis of Variance (ANOVA) was applied to cumulative CO_2 respiration results. The Tukey's post hoc tests were used when needed to provide specific information on which means were significantly different from each other. Changes in pH due to the addition of materials in soil were tested for statistical significance using *T*-tests.

RESULTS AND DISCUSSION

Second incubation of soil samples coming from the wheat field showed generally high respiration rates (Fig. 1). Glycerol incorporated in soil decomposed in all treatments since CO_2 release was always significantly higher than release from soil alone. However, cumulative amounts of mineralized C, which reached a 53% of initial C added (Fig. 1b), were 1.8 times higher when a nitrogen source was added than when soil contained only glycerol. The addition of a small quantity of soil bearing fungi hyphae (inoculum) increased respiration rates in relation to soil containing glycerol only, but the difference between these two treatments did not last till the end of the incubation (Fig. 1a). This figure shows also that inoculum addition did not affect respiration rates in the presence of NH_4NO_3. Mean cumulative CO_2 release at the soil + glycerol treatment was relatively high after the first half of the incubation. This was mainly due to the respiration of one of the three repetitions in this treatment. Despite the elevated respiration rates of this particular sample, no hyphae appeared on the surface of the soil as in the case of the glycerol + inorganic nitrogen treatments.

Third incubation showed again that soil containing an inorganic nitrogen source was much more capable in decomposing glycerol than when glycerol was incorporated alone (Fig. 2). There was no significant difference in respiration between the NH_4NO_3 and the KNO_3 treatment (32 and 34% of initial C mineralized respectively, Fig. 2b). Initial respiration rates during the third incubation were greater than those of the second incubation revealing a shorter initial lag phase for microbial communities to grow in this soil.

Results of the second and third incubation showed that glycerol poses no significant problems to microbial attack and decomposition at least for the particular mixture proportion that was used

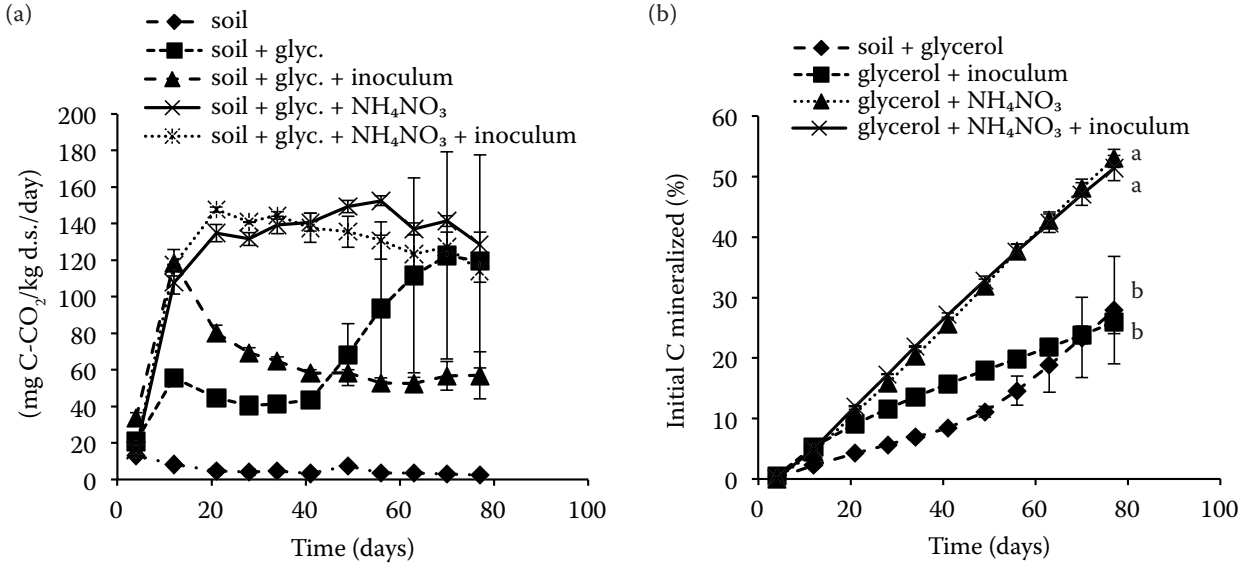

Fig. 1. 2nd incubation (a) daily respiration rates (in mg of C-CO_2 released for kg of dry soil per day) and (b) initial C (%) added mineralized in relation to incubation time when C-CO_2 release from the control treatment (soil alone) was subtracted from each of the other treatments

Error bars show standard error at the mean. Cumulative amounts at the end of the incubation followed by the same letter do not show statistically significant differences between treatments ($P < 0.05$); soil – 75g of soil sampled from a wheat field at the end of autumn, inoculum – small amount of soil taken from a pre-treatment; d.s. – dry soil

in the present study. In 40 days, one third of the organic C in the form of glycerol, methanol, soap and free fatty acids was evolved as CO_2. Moreover, even after 40 or 80 days at the second and third incubation respectively, decomposition did not enter the phase of stabilization, a phase that is usually observed when most added organic material was processed by microbial biomass and dynamics of C mineralization is dominated by the decomposition of dead microbial biomass and microbial secondary products (Voroney, Paul 1984; Thuriès et al. 2001). Improvement of aeration bringing progressively new material in contact with microorganisms and pH drop during the course of decomposition may explain this form of carbon mineralization dynamics.

It is underlined that the mixture proportion that was utilized in present incubations was 5 ml of crude

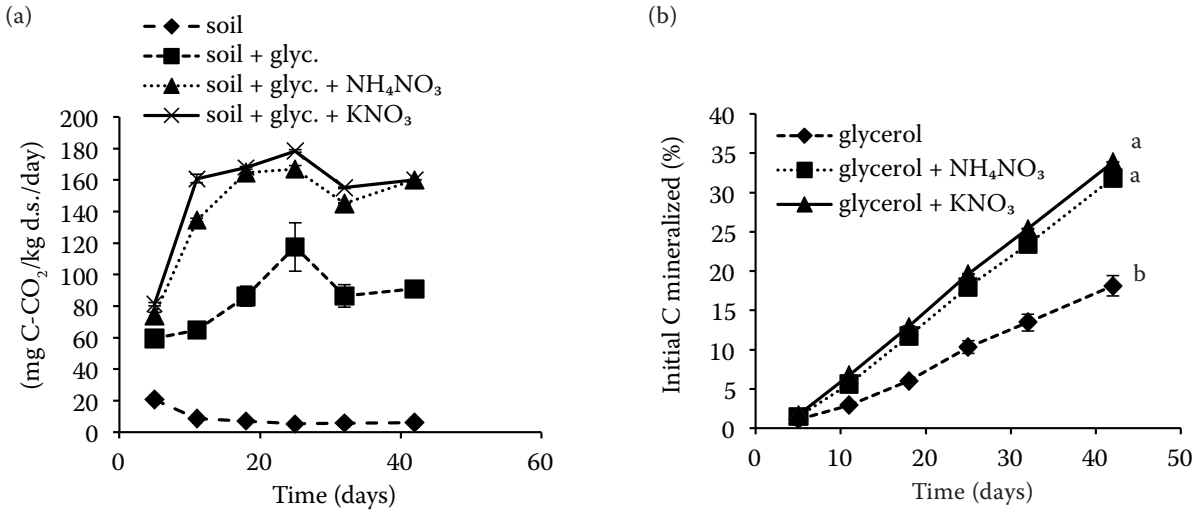

Fig. 2. 3rd incubation results. See caption in Fig. 1

soil – 75g of soil sampled from a wheat field in April; KNO_3 – 1.39 g (0.19 g N); d.s. – dry soil

Table 2. Sample pH values measured before and after the completion of the incubations

Treatments	Initial pH	A	Final pH	B
1st incubation				
Soil	8.10		8.04	ns
Soil + glycerol 5ml	9.36	***	7.87	***
Soil + glycerol 10ml	9.52	***	8.00	***
Soil + NH_4NO_3	7.93	**	7.50	***
Soil + NH_4NO_3 + glycerol	8.71	***	7.42	***
2nd incubation				
Soil	8.29		8.36	ns
Soil + glycerol 5ml	9.35	***	8.07	***
Soil + glycerol + inoculum	9.36	***	8.34	***
Soil + glycerol + NH_4NO_3	8.52	*	7.14	***
Soil + glycerol + NH_4NO_3 + inoculum	8.50	*	7.12	***
3rd incubation				
Soil	7.95		7.88	ns
Soil + glycerol 5ml	9.43	***	8.08	***
Soil + glycerol + NH_4NO_3	8.65	***	7.05	***
Soil + glycerol + KNO_3	9.07	***	7.69	***

values are means of three replications; A – results of the statistical significance analysis (*T*-tests) for the effect of the addition of materials to soil; B – statistical analysis for the comparison between initial and final pH; ns – non-significant

glycerol per 75 g of soil. Assuming a uniform absorption of glycerol at the upper 20 cm of soil having the same bulk density as the one used in this experiment, this proportion corresponds to a spreading of approximately 4 m^3 of glycerol per hectare. Greater amounts of added glycerol may create more unfavourable conditions for carbon mineralization but this is anticipated to affect especially the initial slow decomposition phase by increasing the time lag before the speeding up of microbial development.

The incorporation of glycerol resulted in a significant increase in pH for microbiological pro-

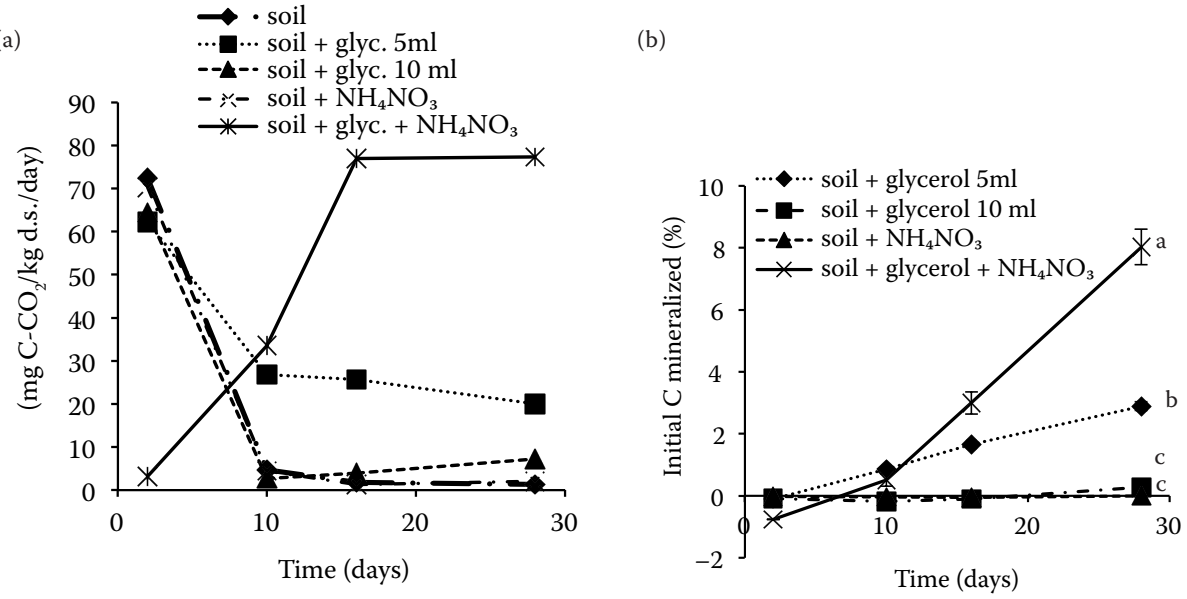

Fig. 3. First incubation results. See caption in Fig. 1

75 g of soil taken from a soil pile; glycerol 5 ml – 5 ml (1.363 g C) of crude glycerol; glycerol 10 ml – 10 ml (2.725 g C) of crude glycerol; NH_4NO_3 – 1.1 g (0.385 g N); d.s. – dry soil

cesses, at least as far as this experiment's glycerol to soil ratio is concerned (Table 2). Sodium hydroxide and soap contained in the crude glycerol phase increased OH^- concentration of the soil solution almost 150 times, reaching levels that are generally considered as restrictive of microbial activity (Alexander 1977). This pH increase was much less when glycerol was incorporated to soil together with ammonium nitrate. KNO_3 mixed with glycerol restricted pH increase caused by glycerol alone but it was not as effective as NH_4NO_3.

Measurements of pH at the end of all incubations were lower than the initial ones. Moreover, the greater the percentage of initial material mineralized the greater the reduction of pH at the end of the incubations. It is likely, that organic acids produced by microbial activity during decomposition of organic materials are capable to neutralize excess alkalinity, reinstating initial pH conditions in the soil solution.

However, what determined glycerol decomposition rates was not only the increase in soil pH caused by glycerol addition, but also the capacity of the microbial community to "restore" initial pH conditions. First incubation showed essentially no response of soil respiration to the incorporation of glycerol alone (Fig. 3). Carbon dioxide was released at a very low rate at the soil + glycerol treatments. This release was slightly higher at the glycerol-5 than the glycerol-10 treatment. The latter did not differentiate from the control treatment except for the last measurement after 28 days of incubation. Nevertheless, respiration rate significantly increased after 10–15 days of incubation at the glycerol + NH_4NO_3 treatment. Higher microbial activity there was also evident from the growth of fungi mycelium on the surface of the soil samples. However, even in this case total C released did not exceed the 8.1% of initial C added (Fig. 3b).

Initial state of soil microbial activity when glycerol was added significantly affected both lag phase preceding the activation of glycerol feeding microbes and subsequent respiration rates. Agricultural soil that was sampled in autumn decomposed glycerol faster than the "inactive" soil of the first incubation, while spring soil, which had been activated by root and residue input during the preceding winter months showed the highest decomposition rates. Soil samples containing presumably low microbial biomasses of the first incubation did not show to be able to decompose glycerol even after 28 days. Present results could not differentiate whether this is due to the inaptitude of soil microorganisms to feed on glycerol or due to their incapacity to withstand high pH conditions following the addition of glycerol. Results from this first incubation indicate also that the greater the amount of crude glycerol that is introduced in the soil the greater the increase in soil pH that is brought about and the greater the time needed before excess hydroxide anions were eliminated. Soil used in this incubation remained without any organic input for more than two years and the easily decomposable fraction of soil organic matter can reasonably be assumed to have been very low.

Given that the provision of N to soil microorganisms relies on the mineralization of N from this fraction of organic matter, the low availability of N could partially or exclusively explain the observed small decomposition rates of glycerol in the first incubation. The activity of microbial biomass and/or the availability of labile organic forms supplying N could equally be called upon the generally greater degree of carbon mineralization in second and third incubation. This is presumably why the addition of ammonium nitrate increased decomposition rates at all incubations providing the necessary N source for the building up of the microbial biomass. The KNO_3 treatment of the third incubation supported the hypothesis that, at least for the conditions of this experiment, N availability was more important than pH in controlling mineralization rates.

CONCLUSION

Although, the results of this study cannot provide evidence on the long term impacts of glycerol addition on soil quality and particularly those concerning an eventual accumulation of sodium, it could be concluded that crude glycerol application to soil could be used as a means to stimulate microbial activity and increase soil organic matter. High pH can only delay the outbreak of microbial development but secondary products of microbial activity are capable in conditioning the pH soil environment and eliminate excess hydroxides. Hence, spreading of this material on the soil surface could provide a means of its disposal if no industrial demand or alternative use is available.

The decomposition of glycerol in soil seems to be favoured in cases where (i) measures are taken so as to improve the C-to-N ratio of the applied material, (ii) the organic matter and microbial activ-

ity of the receiving soil is relatively high, and (iii) the mixture proportion of glycerol to soil is not excessively elevated. Since organic matter, microbial activity and nutrient status are generally not limited in compost piles, addition of glycerol as a composting material may also be considered as a feasible alternative. Recommended dose of the biodiesel by-product named as crude glycerol (a mixture containing different amounts of soap, alcohol, catalyst and water) is approximately 4 m^3 per hectare. Greater amounts of added glycerol may create more unfavourable conditions for carbon mineralization unless applied to acid soils with high content of soil organic matter.

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