Characteristics of compost obtained from winemaking byproducts

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Abstract

A model procedure for the sustainable management of plant biomass related to wine production, namely vine branches from agricultural practices in the vineyard and marcs remaining after grapes crushing, was devised. An artificial humification process was set up that could respond to the needs of environmental sustainability and could be a safe way to be reintroduce in the vineyard part of the organic matter previously exported, thus contributing to recover or maintain vineyard soil fertility. Two different strategies for composting were tested, namely a static pile, made by branches and marcs, and a pile that was fed twice a year alternatively with vine branches and grape marcs. The experimentation lasted 710 days, during which environmental parameters, i.e. temperature and rainfalls were monitored. Growth dynamics of the principal functional groups of microorganism were followed. A characterization of the composted material was obtained by measuring several parameters among which, pH, carbon, nitrogen, sulfur and heavy metals content. The characteristics of the produced compost fulfill the requirements prescribed by the Italian legislation regarding the use of compost as soil amendment. Germination tests demonstrated the absence of phytotoxicity and conversely evidenced a stimulating activity towards root development.

Keywords: organic matter, compost, vineyard, grape marcs, pruning residues, microbiota

1. Introduction

Wine production in the European Union is rather relevant and in 2015 accounted for about 60% of the world production, including Italy, France and Spain being the main producers [1]. Such high levels of production determine the creation of large amounts of residues, namely vine branches from winter pruning and grape marcs from grape pressing for winemaking.

Pruning residues represent a problem for vineyards and at the same time a production cost [2]. Until today the disposal of such wastes follows two main routes: shredding in the field between the rows with possible burial, or branch collecting and burning.

Shredding followed by landfilling can be useful in healthy vineyards since in this case shoots do not constitute potential sources of infection or spread of diseases. On the contrary, this practice may represent a phytosanitary problem in case of vineyards affected by diseases such as *Phomopsis* cane and leaf spot (vine escoriosis) favoring the spread of root rot. In many cases shoots are collected and burned close to the fields but currently this solution is banned for its negative environmental returns in terms of air quality, related to emissions of fine particles, and as a precaution to prevent fires.

Grape marcs are constituted by the solid parts (skins, seeds and sometimes stalks) that remain after grape pressing. They represent a material rich in phosphorous, potassium and magnesium and potentially bioactive compounds [3] but also present some disadvantages, such as low pH (< 4), high C/N ratio (25-40) and presence of potentially phytotoxic compounds (e.g. ethanol, acetic acid, lactic acid, polyphenols). Grape marcs can be processed in distilleries to produce alcohol or alcoholic beverages, while grape seeds can be used for extraction of oil and hulls can be used as supplement for animal feed [4].

Composting is becoming an ecological and economical alternative for reusing plant biomass residues [5] and residues from vitiviniculture, such as pruning residues and grape marcs, can be exploited for production of compost.

The microbiota of grape marcs has been studied by classical microbiological techniques [6 - 8] and by metagenomic approaches [9]. The abundant presence of bacteria and yeast provides a strong stimulus to trigger microbial transformations. It has also been reported that yeasts present in marcs possess a wide range of metabolic activities useful for marc transformation [10].

To date several agronomic residue types have been tested for producing compost. In particular, grape marcs [11- 15] and branches [16] have been separately composted, also with addition of nitrogen sources, such as manure [17].

This work represents the first attempt to combine both kinds of residues coming from vine cultivation and wine production to produce a compost. Microbial dynamics during the entire composting sequence are studied along with physicochemical parameters characterizing the process and the final product. The proposal to produce compost starting from these materials is not particularly new, but the novelty proposed is to try to combine these materials together, also taking into account that they are produced in periods of the year quite apart (branches around February and marcs around September) and also to establish whether it could be possible to install a continuous composting procedure that could be fed twice a year, alternatively with marcs and branches. This composted material could then be periodically removed in part from the pile and used in the field as soil amendment, while the remaining part represents the “inoculum” for the fresh materials added.

2. Materials and methods

2.1. Site description

The study was carried out on a vineyard located in the "D.O.C. Piave" zone (lat. 45° 57' 36" and long. 12° 22' 21") at about 83 m above the sea level, a few kilometers from Cordignano (TV) in the Veneto Region, Italy. The site presents climatic characteristics attributable to warm temperate values and

belonging to the mesothermal climate. Regarding temperatures, the annual average is 16.1 °C with peaks above 23 °C in summer and below 2 °C in winter. The coldest month is January with average monthly temperature of 4 °C and minimum around 0 °C. The warmest months are July and August with peaks that often exceed 30°C and can reach 37 °C.

The average annual rainfall is around 1250 mm, the wettest months are October and November during which rainfalls account for one third of the annual amount. Precipitations are generally abundant during spring (March and April) and scarce in winter. In percentage, the total rainfall is spread 50% in the three autumn months, 25% during the spring, 15% in the months of July-August and the remaining 10% in the winter months. Meteorological data were obtained from the Regional Environmental Protection Agency (Veneto, Italy)

2.2. Composting piles setup and sampling

On September 2011, two static piles, hereafter referred to as pile 1 and pile 2, were set up on a field following the criteria indicated by Finstein et al. [18] by mixing 3 t of grape marcs coming from crushing of grapes of Glera vine variety with 3.5 t of mechanically-shredded vine branches (average branch fragments length was from 5 to 10 cm) from the past winter pruning. These materials represent roughly the amount produced from 1 ha of vineyard.

In February 2012 the same amount of fresh pruning residues was added to pile 1 only. In September 2012, 3 t of fresh grape marcs were added to pile 1 only and in February 2013 about 70% of pile 1 was removed and 3.5 t of fresh mechanically-shredded vine branches were added to the pile and mixed with the existing material.

For each pile, starting from September 2011 (T_0), samplings were carried out regularly about every two months by pooling three sub-samples taken from different parts of the composting mass, at different depth and exposure.

Since the study started in September, when only fresh marc was available, to build the first pile vine branches were obtained from material stored from prunings of the preceding winter. Branches were

mechanically ground and mixed with grape marcs. As shown in Fig. 1, pile 1 was periodically fed with marc and branches, while pile 2 was left to stand without any further addition. The evolution was followed during 2 years, from September 2011 to September 2013 by monitoring environmental, chemical and microbiological parameters.

2.3. Environmental parameters monitoring

The temperature of the piles was measured by using two probes equipped with a data logger for each experimental pile, embedded into the pile at a depth of about 50 cm.

The data loggers were programmed to collect the temperature hourly. Data were elaborated using a MicroLab Lite software (ver. 3.6.5, Fourier Systems, USA).

External temperature and rainfall were provided as average daily values by three ARPA meteorological stations (Conegliano, Vittorio Veneto and Gaiarine) located in the site area.

2.4 Compost chemical analyses and germination index

Samples of compost from pile 1 and pile 2, collected at the beginning of the composting and during the whole maturation phase, were collected to determine whether compost characteristics were compliant to the requirements defined by the Italian law for compost quality [19]. Sample dry weights were determined from 100 g of wet sample by leaving the samples at 40 °C for 24 h and then at 105°C for 24 h [20].

Compost C, N and S content were measured using a CNS automatic analyser (Elementar vario MACRO CNS, Elementar Analysen systeme GmbH, Hanau, Germany). Organic-N was calculated by subtracting NH_4^+ (determined by selective electrode, Sevenmulti Mettler Toledo) from total Kjeldahl N [21]. All analyses were performed in triplicate.

Heavy metals were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) (Ciros Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany) preceded by

acid digestion. Inert residues (plastic, metals, glass and stones) and other additional analyses required by the Italian legislation were performed following the analytical method for compost [22].

Germination index (GI, seed germination and root length test) was measured on water extracts by mechanically shaking the fresh sample of mature compost for 1 h (ratio 1:10 sample: distilled water w/v, dry weight basis). Five ml of each extracts were pipetted into a petri dish lined with a Whatman filter paper where 10 cress seeds (*Lepidium sativum* L.) were placed for an incubation period of 48 h at 25° C in the dark. All experiments were performed in triplicate. GI was determined according to Zucconi et al. [23]. If GI is ≥ 60% the compost is defined as "non phytotoxic". Data were subjected to ANOVA and correlation analysis using Statistica 10.0 package (StatSoft Inc., Tulsa, OK) and Fisher's Protected Least Significant Difference was calculated for mean comparison.

2.5. Microbiological analyses

The following media were used to enumerate viable microorganisms according to their functional characteristics:

TSA (Trypticase Soy Agar) medium (Oxoid) was used for total bacterial counts (TC). After sterilization, 200 µg/ml cycloheximide was added to prevent fungal growth.

BC medium (ammonium sulphate 1 g/l, potassium hydrogen phosphate 1 g/l, magnesium sulfate heptahydrate 0.5 g/l sodium chloride 1 mg/l, agar 15 g/l, carboxymethylcellulose 10 g/l) was used for cellulose-degrading bacteria (CB). After sterilization, 200 µg/ml cycloheximide were added to prevent fungal growth.

AIA (Actinomycetes Isolation Agar) medium (Oxoid) was used for enumeration of actinobacteria (AC). After sterilization, 200 µg/ml cycloheximide were added to prevent fungal growth.

PDA (Potato Dextrose Agar) medium (Oxoid) was used for molds (MO). After sterilization, 100 µg/ml chloramphenicol were added to prevent bacterial growth.

FC medium (10 g/l of carboxymethylcellulose (Sigma Aldrich), 6.7 g/l of yeast nitrogen base (Oxoid)) was used for enumeration of cellulolytic fungi (CF). After sterilization, 100 µg/ml chloramphenicol were added to prevent bacterial growth.

For plate count analyses, 20 g of compost were dissolved into 180 ml of 0.85% NaCl solution and shaken for 2 h at 150 rpm on a rotary shaker al 22°C.

Plates were incubated at 30°C for 7 days for mesophilic and at 60°C for 3 day for thermophilic microorganisms. Each sample was analysed in triplicate.

Enumeration of viable *Salmonella* and *Escherichia coli* cells were performed according the microbiological methods for compost analysis [24].

3. Results and discussion

Regarding the weather trend during the study, from September 2011 to September 2013, temperatures remained near the mean values for the region, never exceeded 30°C and went below zero only for few days during winter 2012.

Rainfall was as statistically expected for the region, mostly concentrated during springtime and autumn and scarce during winter and summer.

The temperature inside the two piles presented remarkable differences throughout the whole period, clearly attributable to the different strategies adopted.

In general, every addition of fresh material resulted in a temperature increase inside the piles. In pile 2 the temperature remained above 50°C for about 1 month and successively its trend followed the evolution of the external values, remaining from 2 to 20 °C higher.

Also pile 1 showed a marked increase at the beginning and a high increase at every addition of fresh material, which was more pronounced following the addition of marc, since this material contains much more readily fermentable sugars than vine branches. In fact following addition of branches the

177 temperature rose up to 50°C while marcs supplementation brought the temperature above 60°C with
178 a peak close to 70°C.
179 This temperature increase is of particular importance regarding the inactivation of possible
180 pathogenic microorganisms and weed seeds. It is reported that a period of at least one week at
181 temperature above 55°C, defined as "thermophilic phase", is required to reach a good sanitization
182 action, while temperatures between 45°C and 55°C improve the degradation rate [25]. Regarding
183 sanitization, while both piles reached the requested temperature at the beginning of the study, only
184 pile 2 exceeded again 55°C in correspondence of marc addition, due to its high content of simple
185 sugars. Considering the "thermophilic phase", pile 1 never reached these values even during summer,
186 while pile 2 temperatures, due to fresh material additions, stayed above 45°C for 54% of the time.
187 Hence, both piles guaranteed a good sanitizing action, since pile 1 did not receive new material after
188 its setup and pile 2 underwent a sanitizing step after each addition of new material.
189 The most relevant advantage presented by pile 1 is the longer permanence of thermal conditions
190 favoring the optimal development of composting activities.
191
192 *3.1 Microbiological analyses*
193 The composting process involves the combined action of several microbial species naturally present
194 in the soil and in plant material which transform complex polymeric materials (mainly celluloses and
195 lignins) into compounds of lower molecular weight. Microbial activity is generally high at the
196 beginning of the composting process due to high availability of nutrients (mainly fermentable sugars)
197 and increases when addition of new plant material takes place.
198 Many different techniques are nowadays available to investigate microbial community dynamics
199 during composting, ranging from traditional plate counts and identification of culturable microbiota
200 [26, 27] to novel methods that give information on microbial community composition without
201 cultivation of organisms [28, 29]. However, in spite of the development of new powerful molecular
202 techniques, no single method has proven to be the most reliable for monitoring microbial communities

in environmental samples and the traditional techniques are still considered useful in environmental microbiology [30, 31].

During the two years of composting we chose to monitor the evolution of five different microbial groups, namely total bacteria, cellulose-degrading bacteria, actinobacteria (actinomycetes), molds and cellulolytic fungi. We decided to focus on functional groups rather than sticking to taxonomic groups that are in general less informative from a functional point of view.

Cultivation of each group was performed on specific selective media and growth of each functional group was studied at two different temperatures, 30°C and 60°C, in order to evaluate the thermophilic and mesophilic component of each fraction.

The present level of understanding of microbial community dynamics in composting processes is largely based on studies carried out with traditional methods, such as cultivation on a plate, followed by isolation and identification of bacteria, actinobacteria and fungi [32].

We performed 11 samplings throughout the study, every two months on average, starting one month after piles were set up to allow the enrichment in the appropriate categories (Fig. 2)

3.2 Total counts

Regarding the mesophilic fraction, total counts gave comparable values for pile 1 and pile 2 throughout the whole experiment, showing higher values at the beginning of the period (5×10^9 ufc/g) that progressively decreased in both piles down to slightly below 10^9 cfu/g. The only relevant difference was recorded after the last addition of branches, that produced an increase in pile 1, reasonably linked to nutrient supplementation.

Thermophilic microbiota had an opposite trend with respect to mesophilics. They started from similar values but had a marked decrease at the third sampling in February, that could probably be ascribed to a shock determined by the dramatic temperature drop inside the mass. After the addition of new material they rapidly resumed their growth and remained at high levels for the rest of the period, with a tendency to increase approximating 10^{10} cfu/g. Pile 2 showed the same initial decrease that was

229 then recovered but population levels did not reach values similar to that of pile 1, particularly towards
230 the end when total population clearly decreased. This seems to evidence that addition of new material,
231 determining an increase in temperature and an overall higher mean temperature, favors the
232 development of a thermophilic population.
233
234 *3.3 Actinobacteria*
235 These Gram positive, filamentous, aerobic or anaerobic bacteria are an important component of the
236 composting microbiota for their action on decomposition and also for their biocontrol potential [33].
237 Their initial number was around 10^9 cfu/g at the beginning in both piles.
238 The mesophilic fraction, after a peak close to 10^{10} cfu/g in both piles at the beginning, maintained a
239 steady level around 10^9 cfu/g but towards the end of the period it started to decrease significantly
240 below 10^8 cfu/g. It is known that composting actinobacteria are mostly thermophilic and perform well
241 between 30°C and 60°C, so this decrease seems to involve a non relevant fraction of this group of
242 microorganisms.
243 Thermophilic actinobacteria showed a similar trend in both piles, although in pile 1 their increase was
244 more rapid, corresponding to the first addition of branches. Both piles showed a marked decrease
245 during the first winter, the only period when the external temperature went below 0°C.
246 After addition of marc, actinobacteria showed a slight decrease with respect to the non-fed pile 2.
247 This could be due to the presence of ethanol produced by yeasts present in marcs, but they also eached
248 the same levels towards the end of the period. The level of thermophilic actinobacteria population
249 was always more than 1 log higher than that of the corresponding mesophilic fraction.
250
251 *3.4 Cellulolytic bacteria*
252 This is a heterogeneous category including gram positive and Gram negative bacteria, aerobic and
253 anaerobic, sharing the capability to enzymatically degrade cellulose.

Mesophilic CB had the highest initial value (above 10^9 cfu/g) among all the categories considered and, after an initial peak that brought their presence close to 10^{10} cfu/g they stabilized their number around 10^9 cfu/g throughout the duration of the study in both piles.

Thermophilic CB showed values considerably lower, starting from $5x10^8$ cfu/g and then slightly increased to values slightly below 10^9 cfu/g at the end. This seems to indicate that thermophilic population takes a considerable time to establish.

3.5 Molds

These eukaryotic aerobic microorganisms are among the most diffuse and important agents of degradation of organic substances, particularly plant material, including celluloses and lignins. Being strict aerobes, the amount of this category is in general much less abundant inside the mass.

Mesophilic molds were present at 10^7 cfu/g at the beginning, which is about 2 logs lower than bacteria and, after a moderate increase, they tended to slowly decrease and at the end of the period they stabilized around the same population density of the beginning. Pile 2 showed a more stable behavior during summer 2013, while pile 1 evidenced a decreasing trend during the same period. This could be probably ascribed to temperature increase of the mass of pile 2 following the addition of branches. Subsequently they found the same equilibrium of the undisturbed pile 2 around 10^7 cfu/g.

Thermophilic molds were the least present fraction at the beginning accounting for around 10^5 cfu/g, but showed in both piles a clear progressive increase (contrary to the correspondent mesophilic counterpart) that determined an increase of more than 2 logs throughout the experimental period.

3.6 Cellulolytic fungi

These fungi are capable of degrading cellulose, as confirmed by their growth in a medium having cellulose as the sole energy source. It can be noted that the dynamics of this group, both for the mesophilic and thermophilic fractions, was very similar to that of total molds, thus indicating that the fungal population developing on plant material is composed almost entirely of cellulolytic fungi.

Overall, from the above data it appears that the two composting strategies do not dramatically modify microbial dynamics, but it can be seen that continuous composting in the long run, appears better from the microbiological point of view, particularly concerning the thermophilic fraction, which established after 2 years at higher values. This procedure appears therefore suitable to select a population that can establish at high levels and is not quantitatively influenced by material addition and mixing.

3.7 Analysis of carbon, nitrogen and sulphur content

The results of CNS analysis related to the 11 collected samples (Fig. 3) show the evolution of carbon, nitrogen and of respective ratio and the percentage of sulfur during composting. The first graph shows the classic trend of the process that leads to the increase of nitrogen (% d.m.), the decrease of organic carbon (% d.m.) and then the C/N ratio. This parameter is certainly the most significant because it defines the proper performance of the composting process. If there is shortage of nitrogen, the decomposition of the materials will proceed more slowly, resulting in a slow-down in microorganism action. By contrast with an excess of nitrogenous substances, a release of nitrogen in the form of ammonia occurs. Generally, a C/N ratio equal to 12 is the limit accepted for mature compost [34]. The sulfur instead presented a trend almost constant for the whole duration of the process but was maintained at higher values, even if no significant differences with respect to sulfur values were detected for the second pile. This effect can be explained considering the periodic pruning addition (February) that concerned only one of the experimental piles. In fact in viticulture about 38% of plant protection treatments are carried out against fungal diseases using sulfur-based fungicides, that remain on the woody parts of the plant even after a long time and that have been measured during the analysis of compost samples. In the first pile higher final percentages were identified also for nitrogen and carbon, although the second pile presented substantially the same tendency. The differences

observed between the two piles related to the content of the three studied elements are consistent with the different management applied to the first pile.

3.8 Chemical analyses of compost

Compost characteristics from the two piles are given in Table 1 and are compared with the quality parameters required by the Italian law [19].

All parameters respected the requested limits (Table 1), with the exception of the moisture content that was higher than 50% w/w with respected to the required values. This was due to the fact that periodically, during the curing phase, water was added to the piles with the aim of favoring microbial activity. The absence of *Salmonella* and *Escherichia coli*, i.e. typical fecal pathogens presents in slurries, makes it clear that biomass sanitization, i.e. human pathogens reduction, occurred during the composting processes. The presence of inert materials was below the legal limit. This fact suggests that the biomass purity level of these materials is very high. The mean concentration for each heavy metal compared with samples of vine shoot pruning [35] shows values always lower than the Italian legal limit and confirms the high quality of the final compost.

3.9 Germination test

As it can appreciated even by eye (Fig.4) the addition of compost suspension induced a better root proliferation corresponding to a germination index above 100% for compost from both piles. If compared to the minimum value required by law, that is equal to 60%, this result indicates not only the total absence of phytotoxicity but instead the presence of a beneficial effects towards the plants.

4. Conclusions

A novel approach for the reutilization of residues produced by winemaking-related activities was studied in order to obtain a compost that can be reintroduced in the vineyard from where the plant

biomass came from. Results suggest that it is possible to set up a continuous composting facility by feeding it twice a year alternatively with marcs and vine branches. The characteristics of the products fulfill the requirements of the Italian law for its use as a fertilizer in the field. Such compost does not show phytotoxic activity, rather it shows some stimulatory action on root elongation in vitro. Further studies will be carried out to confirm the presence of positive traits of the compost in the field, by distributing it in a real vineyard.

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476

Parameter	legal limits	**Compost pile 1**	**Compost pile 2**
Humidity	<500 g kg^{-1} FM	592.3 ± 23.1	581.7 ± 33.2
pH	6.0 - 8.5	8.24 ± 0.04	8.04 ± 0.07
TOC	>200 g kg^{-1} DM	287.2 ± 4.1	299.5 ± 6.9
Humic acid + Fulvic acid	>70 g kg^{-1} DM	135.1 ± 2.3	121.3 ± 3.7
TN	g kg^{-1} DM	20.5	19.7
Org-N	>80% TN	88.94 ± 0.7	90.31 ± 0.9
C/N	<25	13.01	12.53
S	g kg^{-1} DM	3.28	1.88
Cd	<1.5 mg kg^{-1} DM	0.04 ± 0.01	0.03 ± 0.01
Cr^{VI}	<0.5 mg kg^{-1} DM	<0.4	<0.4
Hg	<1.5 mg kg^{-1} DM	0.04 ± 0.01	0.02 ± 0.01
Ni	<100 mg kg^{-1} DM	1.07 ± 0.13	0.98 ± 0.09
Pb	<140 mg kg^{-1} DM	0.87 ± 0.11	0.81 ± 0.21
Cu	<230 mg kg^{-1} DM	26.55 ± 0.33	23.63 ± 0.23
Zn	<500 mg kg^{-1} DM	13.42 ± 0.81	12.92 ± 0.72
Salmonella	MPN absent	absent	absent
Escherichia coli	<1000 UFC/g	absent	absent
GI (*Lepidium sativum* L.)	>60%	108.92	102.66
Plastic, glass, metals (ø>2 mm)	<5 g kg^{-1} DM	0.3	0.1
Stones (ø >5 mm)	<50 g kg^{-1} DM	1.8	0.7

FM: fresh matter; DM: dry matter; TOC: total organic carbon; Org-N: organic nitrogen; TN: total nitrogen; GI: germination index.

Table 1. Characteristics of compost from pile 1 and 2 at the end of the humification process compared with legal limits according to the Italian legislation [19] determined as indicated in ANPA [22].

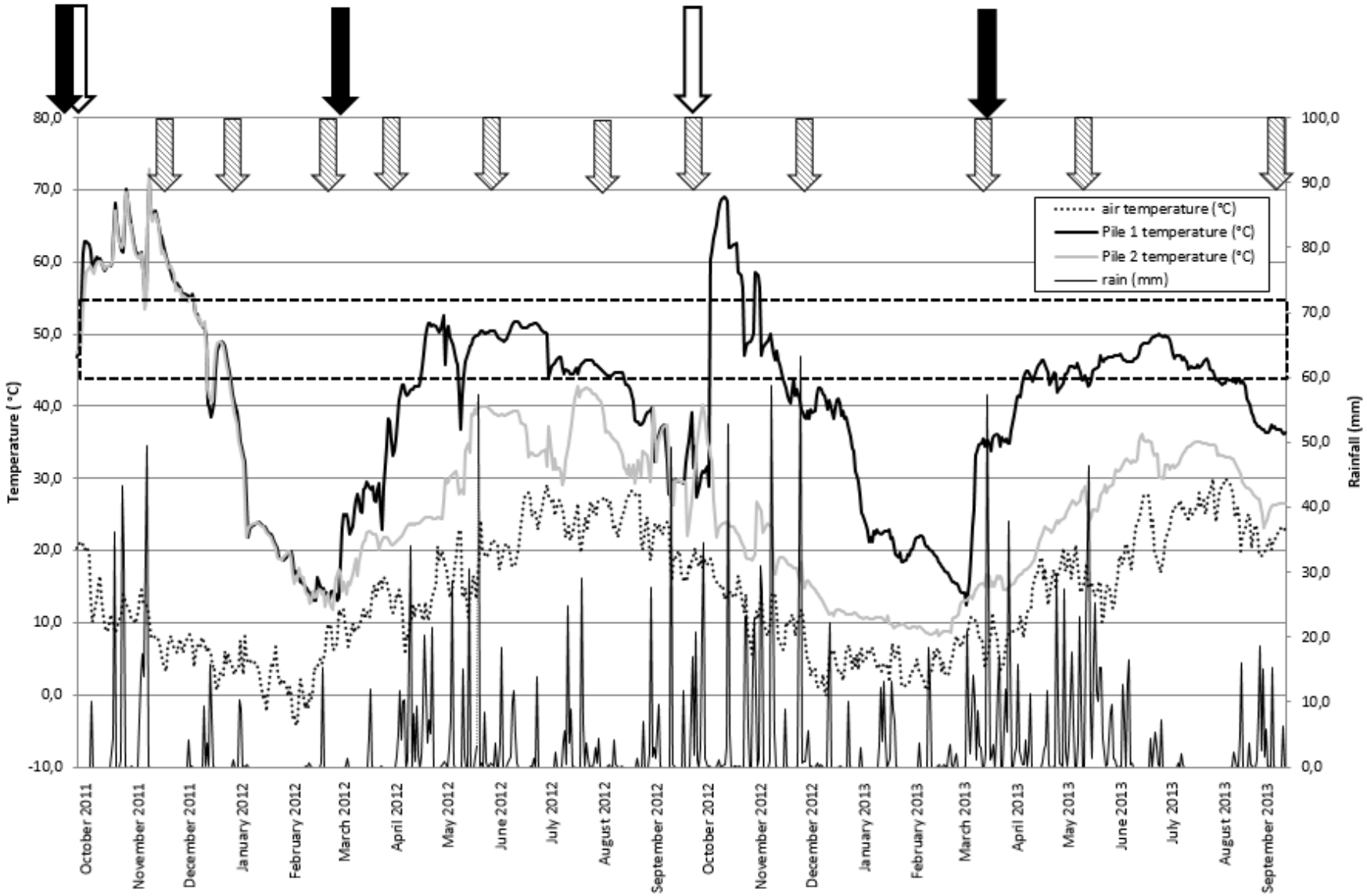
Figure legends

Fig. 1. Trend of temperature and rainfall during composting. Black arrows: vine branches addition; white arrows: grape marcs addition; dashed arrows: sampling times. The dashed region indicates the "thermophilic phase" according to Stentiford [25].

Fig. 2. Microbial populations dynamics during composting. MO: molds; CF: cellulolytic fungi; TC: total bacteria count; CB: cellulolytic bacteria; AC: actinobacteria.

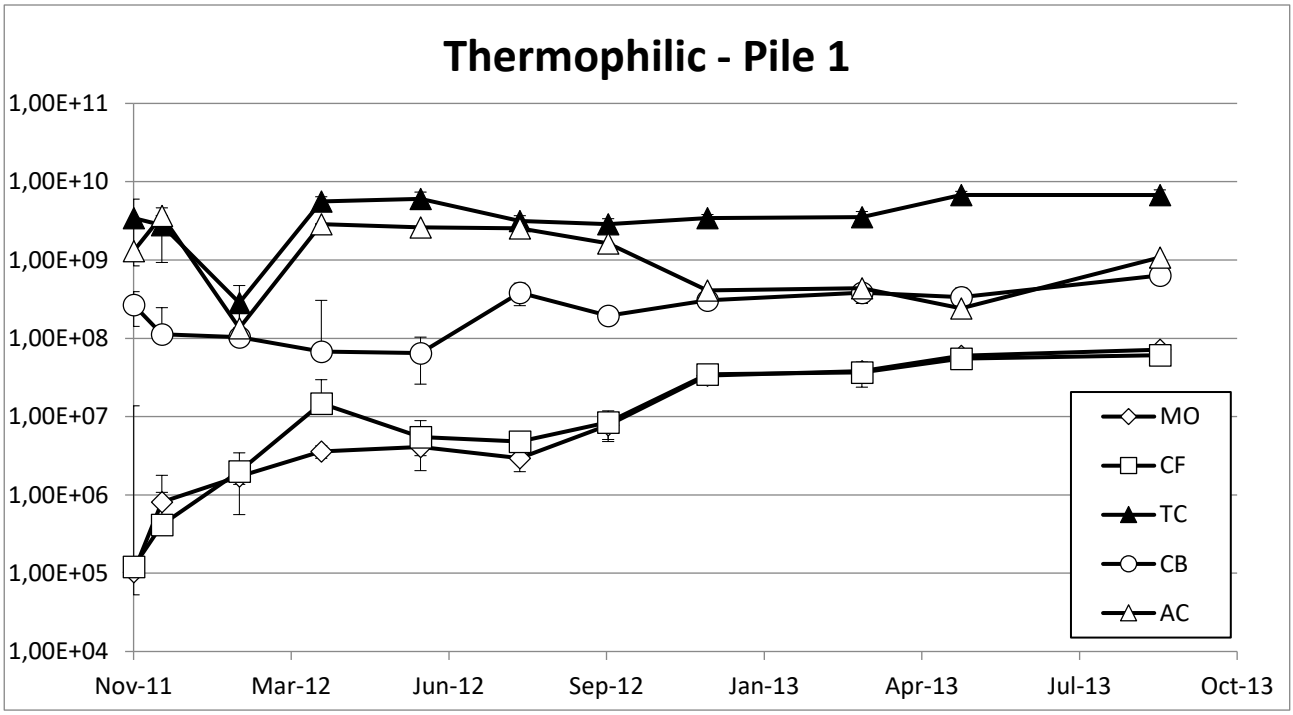
Fig. 3. Percentages of carbon, nitrogen, sulfur, and C/N ratio in the two experimental piles during the humification process. Black: pile1; grey, pile 2.

Fig. 4. Germination test. Seeds in the upper part received compost-shaken suspension, while those in the lower part received 100% water.


air temperature (°C)
Pile 1 temperature (°C)
Pile 2 temperature (°C)
rain (mm)
Temperature (°C)
Rainfall (mm)
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
-10,0
100,0
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
October 2011
November 2011
December 2011
January 2012
February 2012
March 2012
April 2012
May 2012
June 2012
July 2012
August 2012
September 2012
October 2012
November 2012
December 2012
January 2013
February 2013
March 2013
April 2013
May 2013
June 2013
July 2013
August 2013
September 2013

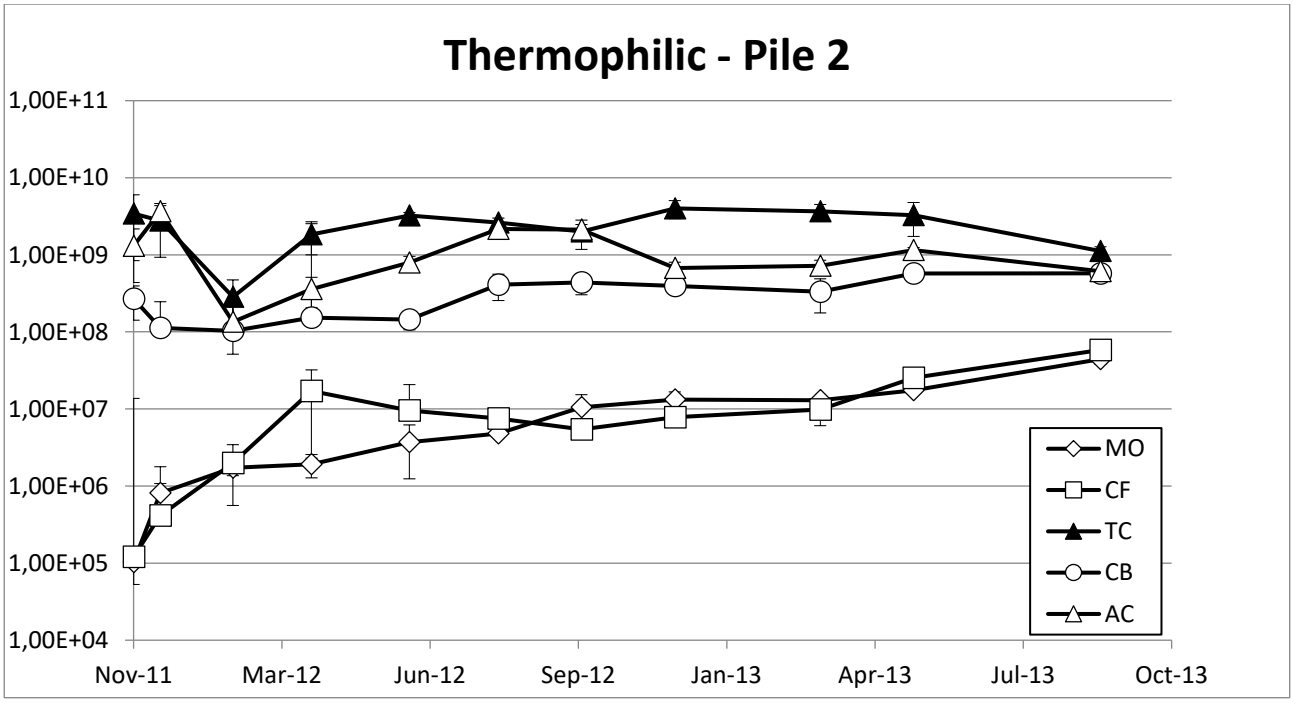
500

501 **Fig. 1.**

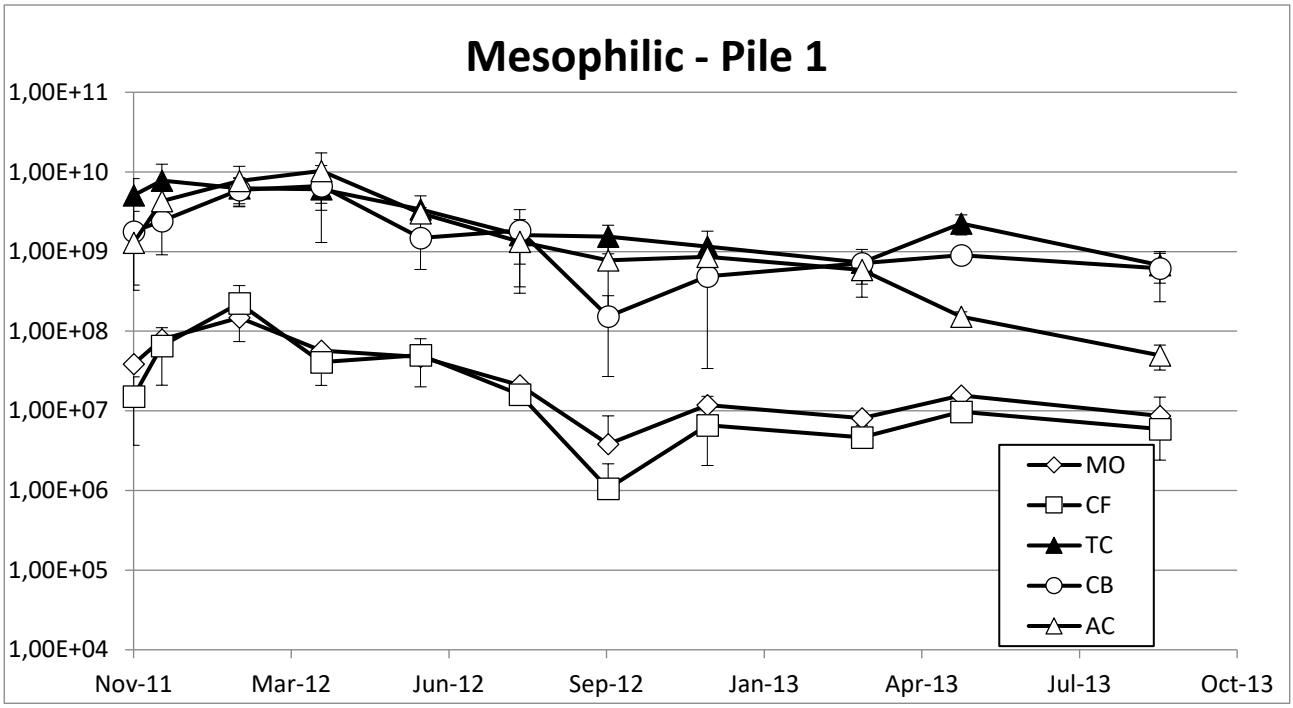

Thermophilic - Pile 1
1,00E+11
1,00E+10
1,00E+09
1,00E+08
1,00E+07
1,00E+06
1,00E+05
1,00E+04
Nov-11
Mar-12
Jun-12
Sep-12
Jan-13
Apr-13
Jul-13
Oct-13
MO
CF
TC
CB
AC

502

503


Thermophilic - Pile 2
1,00E+11
1,00E+10
1,00E+09
1,00E+08
1,00E+07
1,00E+06
1,00E+05
1,00E+04
Nov-11
Mar-12
Jun-12
Sep-12
Jan-13
Apr-13
Jul-13
Oct-13
MO
CF
TC
CB
AC

504



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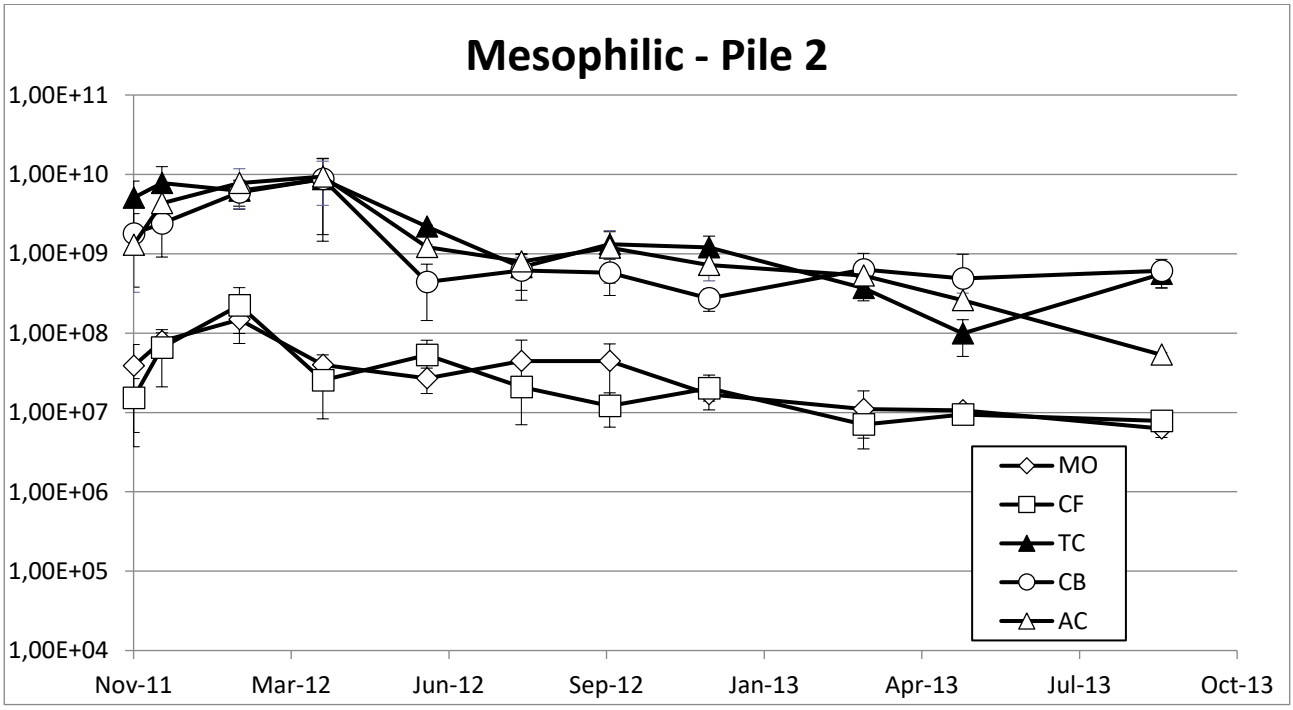
506



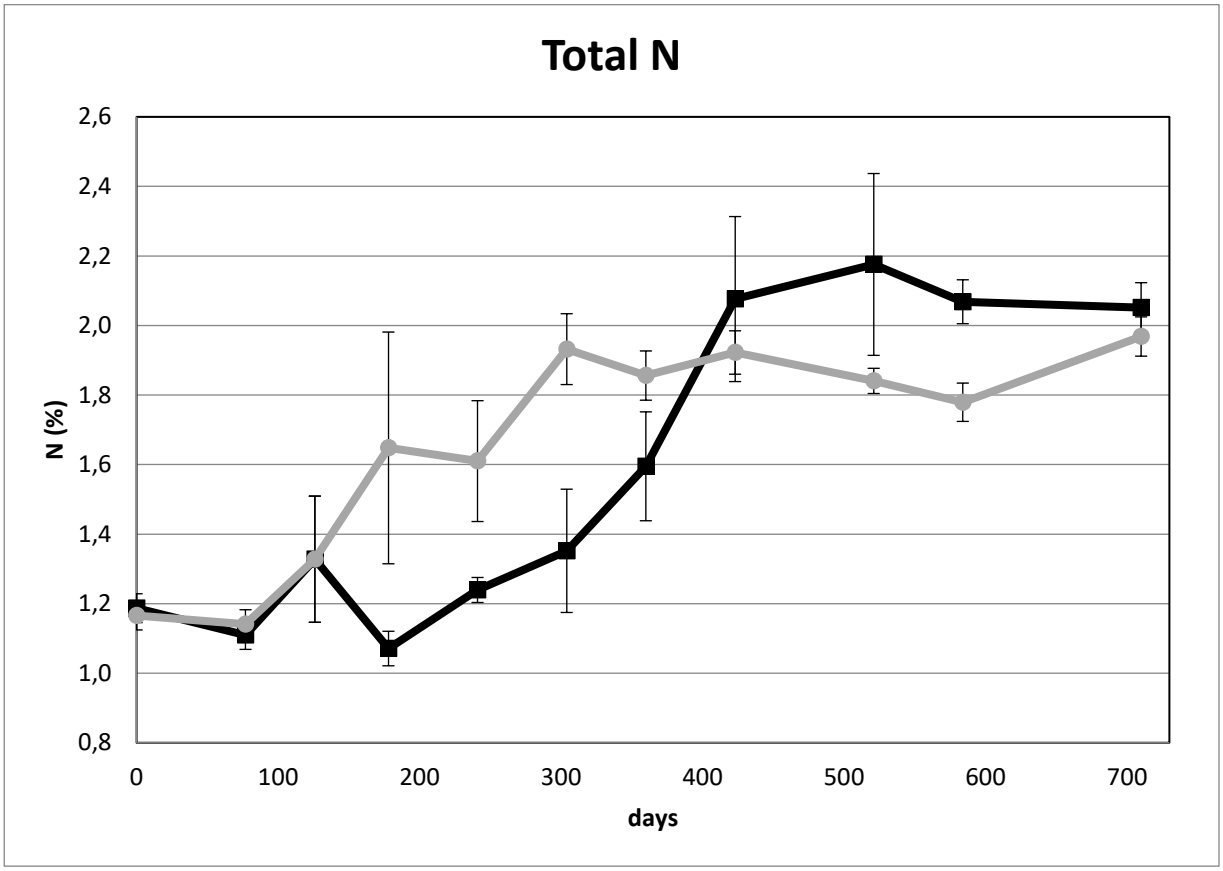
507

508

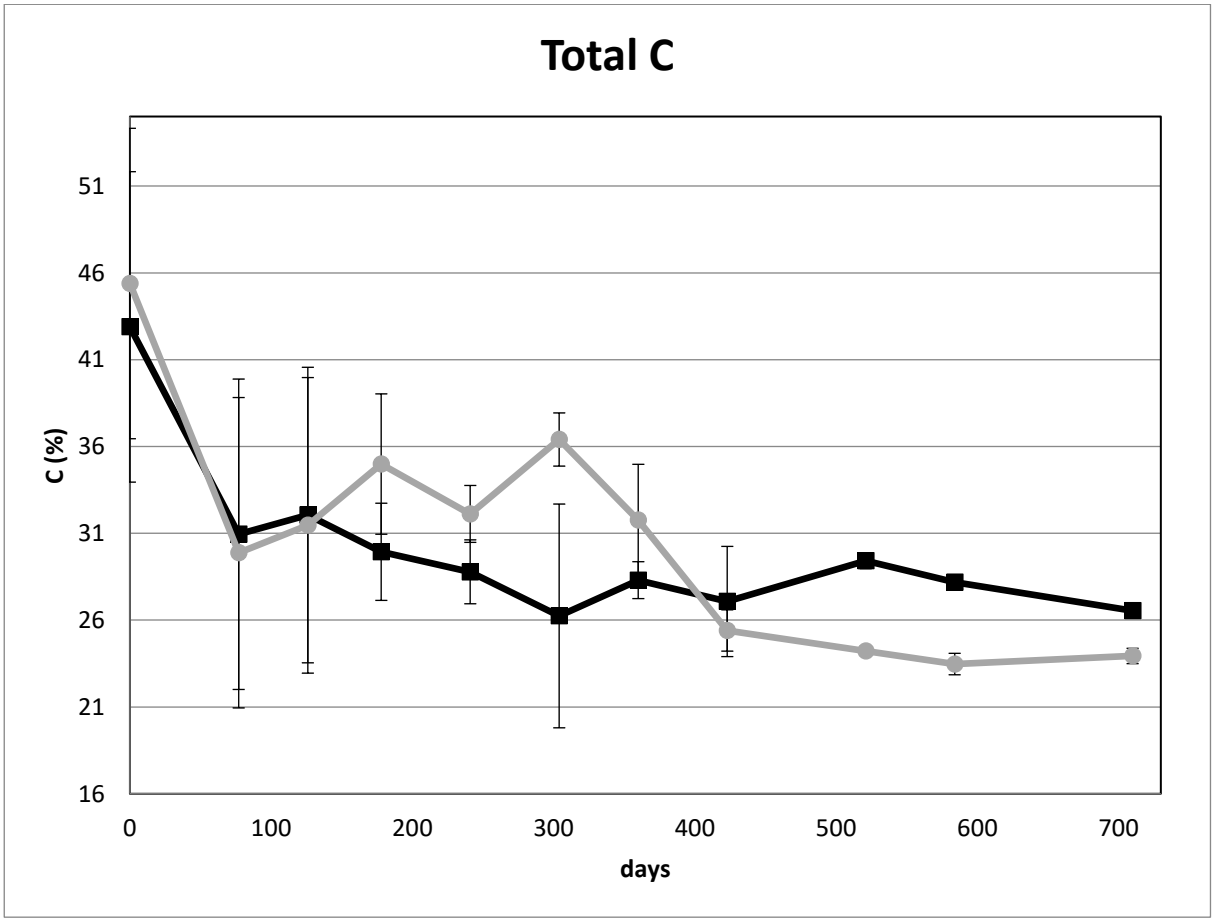
509 **Fig. 2.**

510

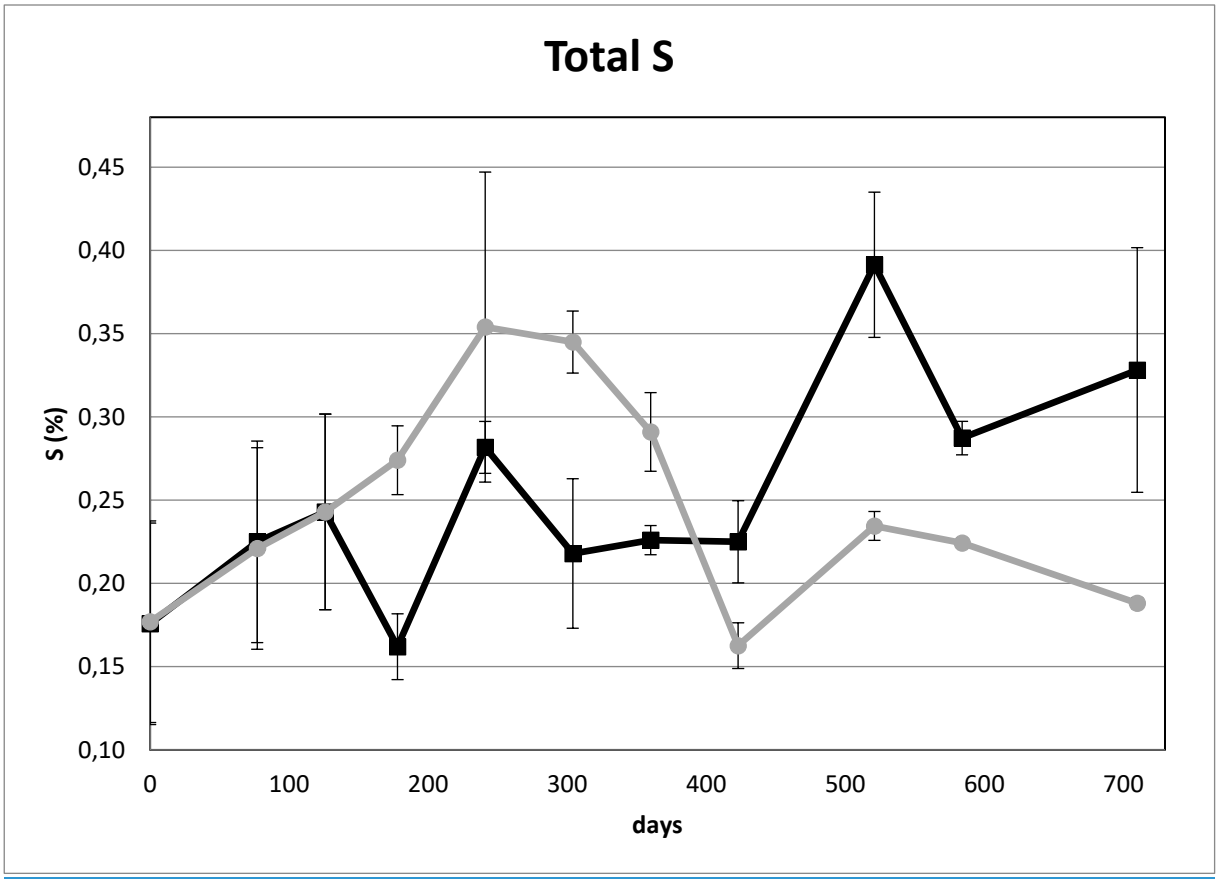
511


Total N
2,6
2,4
2,2
2,0
1,8
1,6
1,4
1,2
1,0
0,8
N (%)
0
100
200
300
400
500
600
700
days

512


Total C
51
46
41
36
31
26
21
16
C (%)
0
100
200
300
400
500
600
700
days

513


Total S
S (%)
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0
100
200
300
400
500
600
700
days

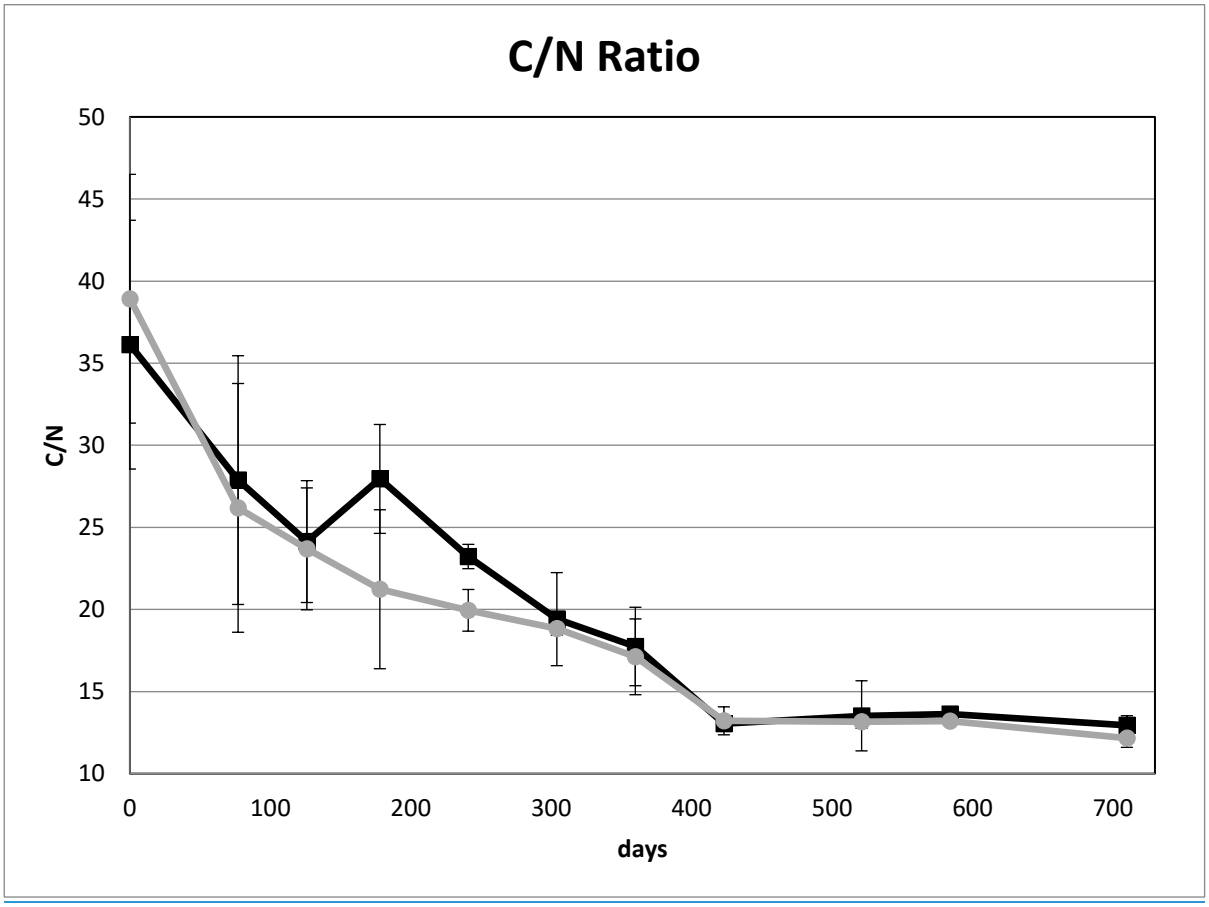

C/N Ratio
C/N
50
45
40
35
30
25
20
15
10
0
100
200
300
400
500
600
700
days

Fig. 3.

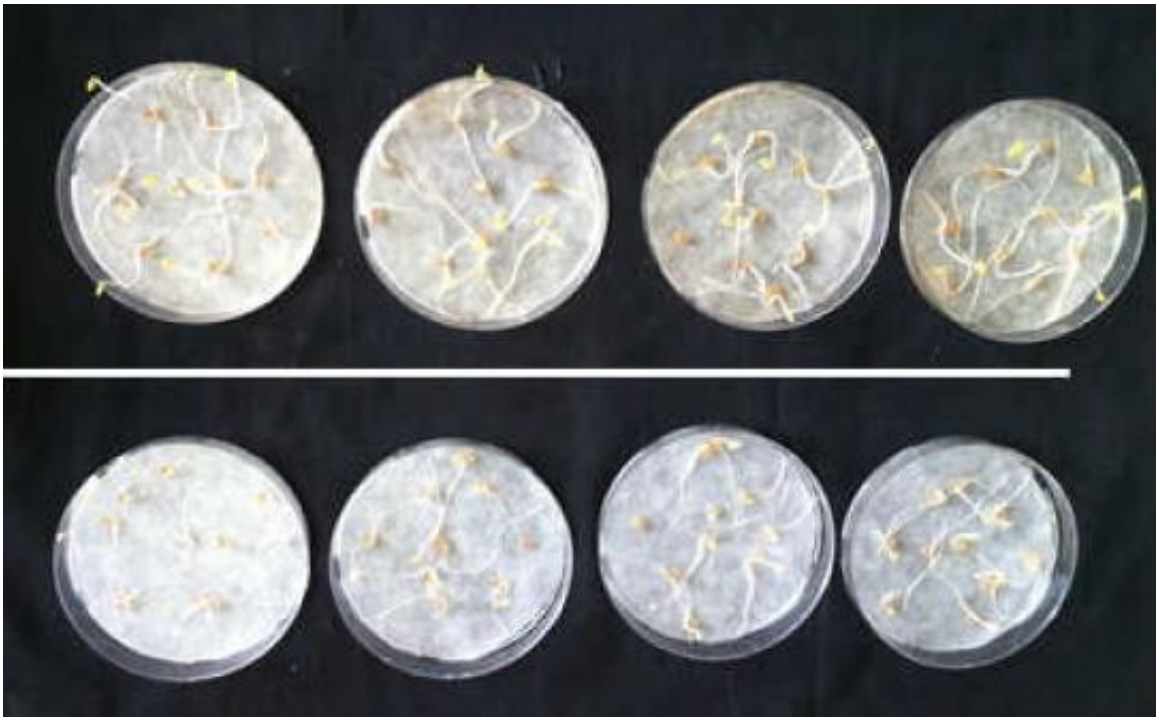


Fig. 4