Dynamics of yard trimmings composting as determined by dehydrogenase activity, ATP content, arginine ammonification, and nitrification potential

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Abstract

Microbial activities, numbers, and biomass are key parameters that can be used to elucidate the dynamics of the composting process. In the present study, four different biochemical parameters (dehydrogenase activity, ATP content, arginine ammonification, and nitrification potential) were measured (1) to monitor the dynamics of yard trimmings composting; and (2) to relate these parameters to changes in microbial numbers, physico-chemical properties, and maturity (stability) of yard trimmings compost. The initial yard trimmings was a mixture of leaves, grass clippings, and shredded bark (1:1:1 v/v). Three windrows, each having a dimension of 1.5 m (height) $\times$ 4 m (length) $\times$ 0.6 m in (width) were established. The temperature profile showed a rapid self-heating of the compost mass from ambient temperature of 20 to 70 °C in the first 24 h of composting. This thermophilic temperature was sustained until day 14 and then dropped to ambient level towards the end of composting (day 63). The maturation of yard trimmings compost was accompanied by a decline in pile temperature to ambient level, increases in bulk density, $(NO_3^- + NO_2^-)$–N, population sizes of actinomycetes and fungi, drop in C:N ratio to 20:1, and decreases and stabilisation of biochemical properties (dehydrogenase activity, ATP content, and nitrification potential) to low levels. Dehydrogenase activity, ATP content, and nitrification potential depended on the changes in C and N content during composting. These parameters were correlated with the populations of either total aerobic heterotrophs or actinomycetes, indicating the usefulness of these parameters as indicators of microbial activity and dynamics of the composting process. Arginine ammonification was dependent on the change of neither C nor N. It also did not correlate with microbial numbers, suggesting that this parameter may not be suitable for evaluating microbial activity and dynamics of yard trimmings composting. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Microbial activities; Yard trimmings; Decomposition; Enzyme activities; Windrow composting

1. Introduction

As a biological process, composting involves a myriad of microorganisms [1–3]. During composting, microorganisms convert organic matter into heat, CO_2, H_2O, partial degradation products and new cell material [4]. The starting material is modified by decomposi-tion through a variety of biological and biochemical transformation. The microorganisms decompose the starting material progressively, breaking it down from complex to intermediate and then to simple compounds [2]. The process also involves dynamic changes in temperature, oxygen status, and nutrient availability, as well as spatial gradients of oxygen, moisture, and temperature [5]. These factors would be expected to strongly affect microbial activities and diversity in the compost. For instance, greatest microbial activity is observed at temperatures between 55 and 65 °C [6,7]. However, if temperatures reach 82 °C, microbial activity would be severely impeded [8]. Moisture content is

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another composting variable that affects microbial activities, as it provides a medium of transport of dissolved nutrients required for the metabolic and physiological activities of microorganisms. Very low ($< 30\%$) or high moisture contents ($> 75\%$) inhibit microbial activities due to early dehydration or anaerobiosis [9,10].

Studies on composting have been focused on physico-chemical parameters in an effort to find simple and reliable indicators of maturity and to improve the efficiency of the composting process. Very little is known about the microbiota, which determine the rate of composting, affect the quality of the product, and produce most of the physical and chemical changes in the compost [2]. Microbial activities, numbers, and biomass are key parameters that can be used to elucidate the dynamics of the composting process. Iannotti et al. [11] and Tiquia et al. [10] proposed that the maturity of the compost may be assessed by the microbiological activity of the product, including total microorganism count, respirometric study, monitoring biochemical parameters of microbial activities and analysis of biodegradable constituents.

In this paper, the dynamics of yard trimmings composting was evaluated using four different biochemical parameters: dehydrogenase activity; adenosine triphosphate (ATP) content; arginine ammonification; and nitrification potential. These biochemical parameters have been used as indices of microbial activities in soils [12–16]. The changes of these parameters during yard trimmings composting and their associations with microbial population numbers, and other important composting parameters (i.e. temperature, C and N) are not well understood. Therefore, this study was conducted to (1) monitor the changes in dehydrogenase activity, ATP content, arginine ammonification, and nitrification potential during composting, and to (2) relate these changes with microbial numbers, physico-chemical properties and maturity (stability) of yard trimmings compost.

2. Materials and methods

2.1. Composting set-up and sampling

Yard trimmings were composted in piles at the Kadoorie Botanic Farm, New Territories of Hong Kong. Raw materials contained leaves, grass clippings, and shredded bark (1:1:1 v/v). This mixture was blended on a concrete compost pad and processed through a chipper shredder before piling. The physico-chemical properties of the initial yard trimmings are reported in Table 1. The compost mix was then piled up in triplicate using a tractor loader. Each pile had a dimension of 1.5 m (height) $\times$ 4 m (length) $\times$ 0.6 m

(width). The piles were covered with a black perforated plastic sheet to avoid heat and water loss. The piles were turned on days 21 and 35 using a tractor loader. Air and compost temperature were monitored daily using a temperature probe. The average windrow temperature from each pile was determined by taking at three measurements at the left, middle, and right side of the piles at 0.75 m depth. Water was added during the experiment to maintain a moisture content level of 60%.

2.2. Physico-chemical properties

Yard trimmings samples were characterised for the following parameters: water content (105 °C for 24 h); bulk density [17]; pH (1:10 w/v l: water extract) using a pH electrode; electrical conductivity (EC; 1:5 w/v sample: water extract) using an EC probe; organic C (loss on ignition; 550 °C for 5 h); and different forms of N [18].

2.3. Enumeration of total aerobic heterotrophs, actinomycetes, and fungi

Quantitative estimation of the populations of three different microbial groups (total aerobic heterotrophs, actinomycetes, and fungi) in yard trimmings compost was determined by direct plating on appropriate culture media [19–21]. The serially diluted compost suspension was inoculated on the agar using a plate frequency technique [22]. Each agar plate was divided into eight sections and about 0.1 ml of the compost suspension was dropped on each of the sections. After incubation, any visible growth observed in any of the eight sections was scored positive. The total number of positive growths was counted and the population of microbial organisms in the sample was estimated using the most probable number (MPN) method [23].

2.4. Dehydrogenase activity, ATP content, arginine ammonification, and nitrification potential

The dehydrogenase activity in yard trimmings compost was analysed using a colorimetric method [16]. An ATP kit (Analytical Luminescence Laboratory, Baltimore, MA) and a luminometer (Monolight 1500, Analytical Luminescence Laboratory) were used to determine the microbial ATP of yard trimmings compost. Arginine ammonification and nitrification potential were quantified using the method suggested by Kandeler [13,14].

2.5. Statistical analyses

The mean and standard deviation (S.D.) of three replicates were reported for all parameters measured.

Table 1
Physico-chemical properties of yard trimmings at different stages of composting

Composting time (days)	Bulk density (g km^{-3})	pH	EC (µS cm^{-1})	Total N (g kg^{-1})	Org N (g kg^{-1})	NH$_4^+$–N (g kg^{-1})	NO$_x^-$–N (g kg^{-1})	Org C (g kg^{-1})	C:N ratio
0	272 ± 64.96	9.43 ± 0.20	2.29 ± 0.15	13.60 ± 0.00	10.28 ± 0.00	3.31 ± 0.45	0.01 ± 0.00	406 ± 21.0	30:1 ± 0.01
7	114 ± 12.92	8.55 ± 0.13	–	14.98 ± 3.86	11.52 ± 3.86	3.42 ± 0.78	0.04 ± 0.01	411 ± 20.4	28:1 ± 6.54
14	138 ± 10.96	8.42 ± 0.13	2.10 ± 0.29	17.84 ± 0.48	16.54 ± 0.48	0.65 ± 0.47	0.65 ± 0.37	278 ± 26.4	23:1 ± 2.16
21	225 ± 31.11	8.54 ± 0.12	–	17.05 ± 0.65	16.15 ± 0.65	0.03 ± 0.01	0.83 ± 0.25	393 ± 12.2	23:1 ± 0.44
35	314 ± 52.62	8.24 ± 0.06	1.37 ± 0.09	19.18 ± 1.77	18.19 ± 1.77	0.03 ± 0.01	0.96 ± 0.44	375 ± 17.5	20:1 ± 2.70
49	265 ± 14.15	8.13 ± 0.15	–	20.68 ± 1.46	19.55 ± 1.46	0.03 ± 0.01	1.06 ± 0.42	356 ± 24.6	17:1 ± 1.89
63	339 ± 28.67	8.35 ± 0.25	1.53 ± 0.09	18.74 ± 0.73	17.35 ± 0.73	0.04 ± 0.01	1.35 ± 0.23	368 ± 3.1	20:1 ± 0.99

Mean and S.D. of three replicates are shown. Bulk density and nutrient parameters are based on 105 °C dry weight. EC, electrical conductivity; Org C, organic C.

Correlation coefficient analysis [24] between biochemical parameters (dehydrogenase activity, ATP content, arginine animonification, and nitrification potential), and physico-chemical and microbial population numbers were computed. A polynomial regression analysis [24] was performed to examine the relationship between biochemical parameters, and organic C and total N. Statistical analyses were computed using SYSTAT version 9.0.

3. Results and discussion

3.1. Temperature profile and population of different microbial groups

The temperature profile showed a rapid self-heating of the compost mass from ambient temperature of 20 to 70 °C in the first 24 h of composting. This thermophilic temperature was maintained until day 14. After day 14, the temperature dropped dramatically and started to level-off to about 30–35 °C by day 37.

The changes in population sizes of different microbial groups (total aerobic heterotrophs, actinomycetes, and fungi) are reported in Table 2. The initial Log_{10} MPN per g for total aerobic heterotrophs, actinomycetes, and fungi were 9.40, 8.78, and 7.27, respectively. These numbers decreased dramatically at day 7 when pile temperatures started peaking at 70 °C, but then increased when pile temperatures started to decline (Table 2 and Fig. 1). Fungi were more sensitive to temperature in this study. Most fungi were eliminated at temperatures above 50 °C and were recovered later when windrow temperatures were moderate (45 °C). De Bertoldi et al. [9] reported that fungi normally increase when the remaining substrate in the compost

Table 2
Log_{10} numbers of total aerobic heterotrophs, actinomycetes and fungi during composting of yard trimmings

Composting time (days)	Heterotrophs (Log_{10} MPN per g)	Actinomycetes (Log_{10} MPN per g)	Fungi (Log_{10} MPN per g)
0	9.40 ± 0.20	8.78 ± 0.08	7.27 ± 0.16
7	6.99 ± 0.16	4.99 ± 0.31	3.09 ± 0.03
14	7.31 ± 0.17	6.28 ± 0.12	3.29 ± 0.44
21	7.55 ± 0.24	6.12 ± 0.35	6.55 ± 0.24
35	8.12 ± 0.15	7.16 ± 0.23	7.33 ± 0.25
49	–	–	–
63	7.19 ± 0.21	7.72 ± 0.11	7.17 ± 0.03

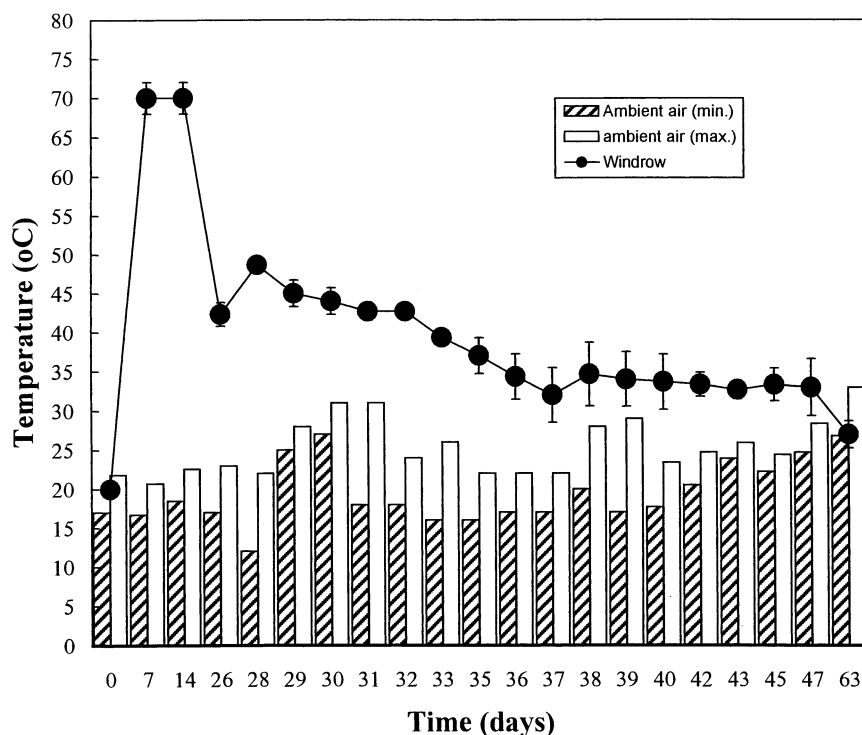Mean and S.D. of three replicates are shown. Data are based on 105 °C dry weight.

Fig. 1. Air and compost temperatures during composting of yard trimmings.

Table 3
Dehydrogenase activity, ATP content, arginine ammonification and nitrification potential during composting of yard trimmings

Composting time (days)	Dehydrogenase (μg TPF g^{-1} 24 h^{-1})	ATP content (μmol g^{-1})	Arginine ammonification (μg NH_4^+–N g^{-1} h^{-1})	Nitrification potential (μg NO_2^-–N g^{-1} 5 h^{-1})
0	1331 ± 53.02	0.15 ± 0.02	0.00 ± 0.00	7.99 ± 1.39
7	1189 ± 68.88	0.47 ± 0.02	1.16 ± 0.76	6.33 ± 3.01
14	908 ± 44.37	0.42 ± 0.01	0.15 ± 0.14	5.08 ± 0.04
21	716 ± 28.31	0.40 ± 0.01	9.82 ± 3.77	3.54 ± 1.77
35	745 ± 80.38	0.33 ± 0.03	3.74 ± 1.70	3.28 ± 1.61
49	714 ± 25.60	0.17 ± 0.02	0.20 ± 0.24	1.11 ± 0.78
63	717 ± 43.90	0.17 ± 0.02	0.05 ± 0.08	1.66 ± 0.63

Mean and S.D. of three replicates are shown. Data are based on 105 °C dry weight.

Table 4
Correlation coefficients between biochemical properties and physico-chemical and microbial properties of yard trimmings

Composting parameters	Dehydrogenase activity	ATP content	Arginine ammonification	Nitrification potential
Temperature	0.26^{ns}	0.72^{**}	-0.001^{ns}	0.22^{ns}
Bulk density	-0.70^{**}	-0.80^{***}	0.04^{ns}	-0.53^{*}
pH	0.79^{***}	0.38^{ns}	-0.13^{ns}	0.69^{***}
EC	0.85^{***}	0.63^{*}	-0.45^{*}	0.75^{***}
Total N	-0.79^{***}	-0.55^{*}	0.05^{ns}	-0.82^{***}
Organic N	-0.86^{***}	-0.50^{*}	0.15^{ns}	-0.83^{***}
NH_4^+–N	0.92^{***}	0.45^{*}	-0.22^{ns}	0.74^{***}
NO_x^-–N	-0.89^{***}	-0.70^{***}	-0.03^{ns}	-0.65^{**}
Organic C	0.74^{***}	0.75^{***}	0.21^{ns}	0.69^{***}
C:N ratio	0.37^{ns}	0.44^{*}	-0.002^{ns}	0.55^{**}
Heterotrophs	0.44^{*}	0.03^{ns}	-0.10^{ns}	0.45^{*}
Actinomycetes	0.13^{ns}	-0.64^{**s}	-0.27^{ns}	0.19^{ns}
Fungi	0.29^{ns}	-0.43^{ns}	0.36^{ns}	0.09^{ns}

Correlations were based on 21 raw data from three piles with the exception of EC and microbial parameters (total aerobic heterotrophs, actinomycetes and fungi) where 12 and 18 observations were taken, respectively. *, **, and ***, indicate significant correlations at 0.05, 0.01, and 0.001 probability levels, respectively; ns, not significant; EC, electrical conductivity; heterotrophs, total aerobic heterotrophs.

are predominantly cellulose and lignin, which normally occurs towards the later stages of composting (curing process). The exact role of fungi during composting is not clear although some of these might be involved in the decomposition of cellulose and lignocellulosic compounds of the compost, and thereby provide more readily available C to the bacteria [4]. Like fungi, actinomycetes also utilise complex organic material. They tend to grow in numbers in the later stages of composting, and have been shown to attack polymers such as hemicellulose, lignin, and cellulose [9]. The numbers of these three microbial groups were positively correlated with a number of important enzymes including β-glucosidase, β-galactosidase, and α-galactosidase during co-composting of poultry litter and yard trimmings [25].

3.2. Dehydrogenase activity

Dehydrogenase activity has been used as a measure of overall heterotrophic microbial inhabitants in soils and composts [10,26,27]. In the present study, the dehy-

drogenase activity was highest (1331 μg TPF g^{-1} 24 h^{-1}) at the beginning of composting period (Table 2). The activity decreased as composting proceeded and began to level-off from day 49 onwards. El-Shinnawi et al. [28] also reported a decrease in dehydrogenase activity during anaerobic digestion of cattle manure. In their study, the immature cattle manure harboured high numbers of active bacteria and as the digestion proceeded, the bacterial numbers decreased. They also observed a decrease in some compounds, especially those preferred by much of the heterotrophic population. The initial yard trimmings had the highest number of total heterotrophs at the beginning of composting and its population decreased with composting time (Table 2). This trend paralleled changes in dehydrogenase activity (Tables 2–4).

3.3. ATP content

ATP content has been considered as a microbial biomass indicator in soil [29]. It has also been used as an indicator of microbial activity and degree of degra-

dation of spent pig litter compost [10]. The ATP content in the piles rose rapidly from 0.15 to 0.47 μmol g^{-1} in the first week of composting (Table 2). As the temperature started to decline, the ATP content dropped continuously to 0.17 μmol g^{-1} by day 49 and maintained at this low level until day 63. The decrease in ATP content in this composting process could be attributed to lowering of microbial activity, since ATP is related to intact microbial cells [30]. ATP stabilisation at day 49 onwards may indicate maturity of yard trimmings when most bacteria have been replaced by specialised groups of microorganisms such as actinomycetes and fungi, which are characterised by slower metabolism and hence slower ATP production. Similar observations were found by Garcia et al. [31] on composting of organic wastes. The ATP content of yard trimmings was positively correlated with temperature and was negatively correlated with actinomycete numbers in this study (Table 4), suggesting that this parameter can be used as an indicator of microbial activity during composting of yard trimmings.

3.4. Arginine ammonification

Ammonification of arginine is a common process in microorganisms [32]. Arginine mineralised to ammonium during decomposition can be readily extracted from soil or composts and measured [33]. Alef and Kleiner [34] suggested that arginine ammonification was proportional to the soil microbial biomass and may be used as an indicator of microbial activity of yard trimmings compost. Ammonium–N mineralised from arginine increased from non-detectable levels to 1.16 μg NH$_4^+$–N g^{-1} h^{-1} by day 7 (Table 3). Maximum ammonium–N production was observed (9.82 μg NH$_4^+$–N g^{-1} h^{-1}) at day 21 when pile temperatures started to decline (Fig. 1). Ammonium–N production began to decline after day 21 and reached a low value of 0.05 μg NH$_4^+$–N g^{-1} h^{-1} at the end of composting (Table 2). Alef and Kleiner [32] reported that most heterotrophic bacteria are able to ammonify arginine. Interestingly, arginine ammonification did not show any correlation with total aerobic heterotroph counts (Table 4), and may not be used as a reliable indicator of microbial activities in yard trimmings composting.

3.5. Nitrification potential

Chemoautotrophic bacteria are reported to be largely or solely responsible for nitrification at a pH above 5.5 [35]. Various methods have been used for the determination of nitrification [36–38]. Total numbers of autotrophic ammonium oxidisers has been counted with the MPN method and fluorescence antibody technique. In many cases, incubation experiments were performed [14]. In the present study, nitrification potential was used to assess the dynamics of the composting process in terms of the conversion of inorganic or organic N of yard trimmings from a reduced to a more oxidised state. The nitrification potential of yard trimmings compost as indicated by NO$_2^-$–N production during a 5 h incubation, decreased with composting time from initial value to 7.99 to 1.66 μg NO$_2^-$–N g^{-1} 5 h^{-1} (Table 3). Changes in nitrification potential paralleled the change in dehydrogenase activity (Table 3). Nitrification potential was positively correlated with total aerobic heterotrophs and other important compost parameters such as pH, EC, NH$_4^+$–N, organic C and C:N ratio (Table 4), indicating that nitrification potential may be used as an indicator not only of microbial activity but also as a parameter to assess the composting process.

3.6. Relationship between biochemical parameters and C and N

Results of the regression analysis showed a linear relationship between C and dehydrogenase activity ($r^2 = 0.54$), ATP content ($r^2 = 0.56$), and nitrification potential ($r^2 = 0.48$; Fig. 2A, B and D), indicating that these biochemical properties are dependent on changes in C content. The changes in these three biochemical parameters are also dependent on the change of N during composting (Fig. 2E, F, and H). During composting, the degradable organic matter and nitrogenous compounds in the yard trimmings were broken down by microorganisms. This process resulted in increases in microbial activities. Towards the end of composting, no further decomposition is taking place as the C and N became stabilised. Consequently, no more heat is released as a result of microbial activities, and so the dehydrogenase activity, ATP content, and nitrification potential of yard trimmings dropped and stabilised to low levels. Arginine ammonification was dependent neither on the change of C ($r^2 = 0.04$) nor N ($r^2 = 0.002$) (Fig. 2C and G), suggesting that this parameter may not be suitable for evaluating the dynamics of yard trimmings composting.

3.7. Evaluation of compost maturity

Most of the criteria used in the evaluation of the composting process, compost stability (maturity) and quality were based on physical and chemical parameters of the organic material, whose behaviour reflects the metabolic activity of microorganisms involved in the composting process [9,39–41]. These parameters

include a drop in temperature, degree of self-heating capacity, oxygen consumption, phytotoxicity assays, cation-exchange capacity (CEC), organic matter and nutrient contents, and C:N ratio.

The maturation of yard trimmings compost was accompanied by a decline in pile temperature to ambient level, increases in bulk density, $(NO_3^- + NO_2^-)$–N, population sizes of actinomycetes and fungi, drop in C:N
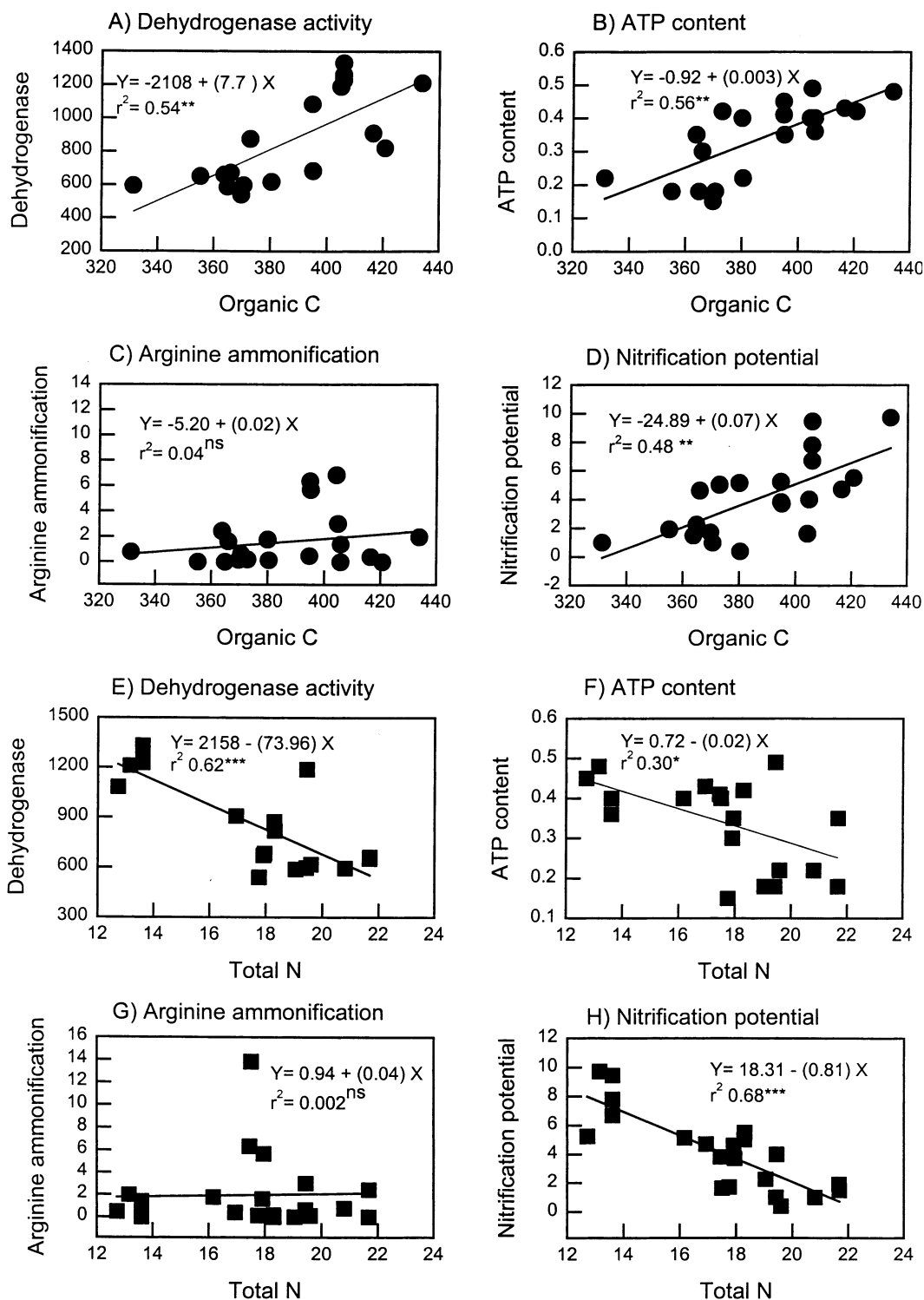

Fig. 2. Relationship between organic C and total N contents with dehydrogenase activity, ATP content, arginine ammonification and nitrification potential. Regression equation, line of best fit and statistical significance are shown. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, significant.

ratio to 20:1, and decreases and stabilisation of biochemical properties (dehydrogenase activity, ATP content, and nitrification potential) to low levels.

Acknowledgements

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