This is a pre-print of an article published in Waste and biomasss valorization (Ed. Springer). The final authenticated version is available online at: https://doi.org/[10.1007/s12649-016-9532-2

Biodegradation activity of eight organic substrates: A correlation study of different test methods

Alexandros Evangelou[a], Paolo Calabrò[b], Rosa Greco[b],

Antoni Sánchez[c], Dimitrios Komilis[*,a, c]

[a] *Laboratory of Solid and Hazardous Waste Management, Department of Environmental Engineering, Democritus University of Thrace, Xanthi, 671 32, Greece*

[b] *Department of Civil, Energy, Environmental and Materials Engineering, Mediterranea University of Reggio Calabria, ReggioCalabria, 89122, Italy*

[c]*Composting Research Group(GICOM), Department of Chemical Engineering,UniversitatAutònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain*

**Corresponding author: Dimitrios Komilis*

E-mail: dkomils@env.duth.gr, Tel.: +30-25410-79391

Abstract

The biological activity of eight organic substrates was assessed using different techniques under aerobic and anaerobic environments at different scales. The used substrates included simulated fresh to actual stabilized composts. Experiments included dynamic respiration (with 30 L and 5.5 L reactors), static respiration (with 1 L reactors) and biochemical methane potential (BMP) tests (with 1.1 L flasks). The indices evaluated were: the 24-h dynamic respiration index (DRI_{24}) and the cumulative dynamic respiration indices at 4 and 7 d ($DCRI_4$ and $DCRI_7$, respectively) measured with two different methods, the maximum dynamic CO_2 generation rate ($D\text{-}CO_2\_24$) at 24h and a dynamic cumulative CO_2 generation after 7 days ($D\text{-}CO_2\_7$), the maximum static O_2 consumption rate in 12h and 24h (SRI_{12} and SRI_{24}), the static cumulative O_2 consumptions after 4 and 7 days ($SCRI_4$ and $SCRI_7$) and the static CO_2 generation after 7 days ($S\text{-}CO_2$) and the BMP after 30 days. The 24-h dynamic respiration index (DRI_{24}) ranged from 25 to 3000 mg O_2 $kg^{-1}VSh^{-1}$ in one lab and from 150 to 3500 mg O_2 kg^{-1} VS h^{-1} in the other. A positive statistically significant correlation was achieved between the two types of dynamic indices. In addition, the CH_4 production after 30 d showed a strong positive correlation with both DRI_{24} indices and the cumulative dynamic respiration indices at 4 and 7 d ($DCRI_4$ and $DCRI_7$), as measured in both labs. The static respiration indices did not correlate well with the dynamic respiration ones. The practical implications of the use of the biodegradation activity indices were also analysed and discussed.

Keywords: biochemical methane potential; compost; dynamic respiration index; organic substrate; respiration activity.

1. INTRODUCTION

A wide research on respiration activity indices has been conducted in order to characterize the biodegradation activity and stability of organic substrates [1-10]. Stability usually refers to the resistance of organic matter to biodegradation [11] and/or the extent to which readily biodegradable organic matter has decomposed [12]. A high content of an easily degradable fraction means a low biological stability and vice versa [13]. Stability is commonly assessed via measurements of oxygen consumption and carbon dioxide generation [4, 12, 14-15], methane generation [15-17] as well as via temperature evolution through the self-heating test [18-20]. All above techniques can be adopted to measure the biodegradation activity of fresh to stable substrates.

In the current study aerobic microbial respiration activity (in which O_2 consumption and CO_2 generation are the dominant indices) and anaerobic activity (in which the CH_4 generation is the key index) were measured. These biological indices are essential for evaluating biomass degradation activity for compliance or process control purposes, since these methods, although in a smaller scale can reproduce the actual waste degradation process [21] and can designate treatment technologies.

In particular, with regard to the aerobic respiration activity, different aeration modes have been tried; static with intermittent air flow and dynamic with continuous air flow. In dynamic respiration methods, further differences exist regarding the selected airflow rate. Airflow rates between 20 and 30 ml min^{-1} have been reported by [9, 21] in order oxygen content at the exhaust gases be above 10% (v/v). Similarly, [4] continuously adjusted the airflow rate in order to constantly maintain an oxygen content of 140 ml L^{-1} air (14% v/v) at the reactor's outlet stream.

Experiments in several scales have been reported to assess static and dynamic

respiration indices [3-4, 8, 12, 22-23]. For example, aerobic respiration has been assessed in small 500 mL dynamic reactors [9], in 5.5 L reactors [24] and in up to 30 L reactors, such in the Di.Pro.Ve-CosTech® system [25].

How representatively can all these reactors measure the actual respiration activity of the material? Can they all assess the same microbial activity regardless of sample size and boundary conditions?

Although larger reactors are usually better in terms of sample representation, they are usually more complex during their operation, difficult and costly to be constructed. Moreover, they require large sample sizes and replication can be sometimes very limited to non-existent due to cost constraints. On the other hand, smaller reactors are easier to construct and operate and allow the experimenter to run simultaneously many replicated experiments. The Di.Pro.Ve-CosTech® system is more automated and allows the measurement of several variables at short time intervals; in addition, air flow can be controlled either through the oxygen content or through the temperature readings. However, due to economical limitations in several waste treatment facilities, plant operators are seeking less cost-benefit techniques to assess biodegradation activity and compost stability. Stability indices are used for compliance tests in Germany at a national level [26], in Italy at regional/provincial level [27] and in England and Wales [28], for both MBT materials and composts. Moreover, DRI has recently received official recognition at EU level for purposes of standardization [29] and validation [30].

The objective of this work refers to i) assess the biodegradation activity of 8 organic substrates of different biodegradabilities in two different laboratories, using different microbial activity assessment techniques per laboratory and ii) investigate correlations among those indices. One laboratory is located at the Mediterranea

University of Reggio Calabria (Italy), and will be herein referred to as either UNIRC or ITA, whilst the other is located at the Democritus University of Thrace in Xanthi (Greece) and will be herein referred to as DUTH or GRE.

2. MATERIALS AND METHODS

2.1. Substrate description and preparation

Eight different organic substrates of different origin and composition were investigated in this study (Table 1). Samples were selected in such a manner so that to cover a wide range of organic matter contents and expected degradabilities.

HC_1 was a mature home compost obtained from household organic waste, produced by kitchen and garden waste mixed with a limited quantity of wood combustion residues, constituted by coal and ash. Sampling for that substrate took place after one year of composting in a custom made plastic composter, by opening the top cover and grabbing randomly samples from several heights of the composter. HC_2 was a 21-d aerobically stabilized home compost produced in Italy from a mixture of kitchen waste (60% wb) and wood chips (40% wb). OFMSWC was a compost prepared from the organic fraction of commingled municipal solid waste (OFMSW) and was obtained from a full scale mechanically and biologically treatment (MBT) facility at Reggio Calabria after 28 days of aeration. Approximately 10 kg of material was randomly sampled by the plant managers.

Substrates SIM_FW1 and SIM_FW2 were raw food wastes that were artificially prepared in both labs on-site. This was done in order to achieve an expected high degradability and to check the reproducibility of measurements between the labs with those simulated wastes. SIM_FW1 was prepared by mixing 25% of cooked pasta, 25%

of white bread, 25% of chopped apples and 25% of grilled minced beef meat (on a wet weight basis, wb). SIM_FW2 was prepared by mixing 50% of uncooked pasta, 30% of uncooked frozen fries and 20% of beef dog food (all in wb). Those ingredients were obtained from the same commercial chain that is located in Italy and Greece. The two aforementioned substrates (SIM_FW1 and SIM_FW2) were left for a week in a sealed plastic bag after preparation, and before the initiation of the experimental runs, so that to better simulate actual food wastes. FORW was a sample of leaves obtained from a forest floor during spring, by collecting all the available leaves from a 2 m^2 randomly selected area. FAMN was a fresh (1 week old) animal manure, collected from a cow breeding facility, that consisted of cow manure and straw. AMNC was obtained from the same facility, after passing through a solid-liquid separator and after stored in piles for 1 week.

For substrates HC_1, OFMSWC, FORW, FAMN and AMNC almost 4 to 10 kg (wet weight) were collected, while sampling was followed by a sequential quartering process in order to ensure randomness and homogenization. In the case of simulated substrates (SIM_FW1 and SIM_FW2) and HC_2, the whole amounts were used for the analysis.

Insert Table 1

HC_1, HC_2, OFMSWC and FORW were collected from Reggio Calabria (Italy), and then divided and individually sealed in two different plastic bags, containing approximately the same substrate quantity (about 4-10 kg). One of the two bags was placed in a non-insulated paper box, that was also thoroughly sealed and was shipped by

139 airmail to Xanthi (DUTH, Greece). The other bag was kept in UNIRC at room
140 temperature until the other part reached Greece, usually after 4-5 days. As soon as
141 samples arrived in Greece, characterization started at the same time in both labs. FAMN
142 and AMNC were collected from a cow breeding plant near Xanthi (Greece) and were
143 shipped to Italy following the same abovementioned procedure. Substrates SIM_FW1
144 and SIM_FW2 were prepared concurrently in both laboratories, using the same raw
145 materials and proportions, right before the initiation of the analyses.
146
147 *2.2. Initial characterization*
148 The preliminary characterization of the substrates was performed simultaneously
149 at both labs using similar standard techniques. Measurements of moisture (M), volatile
150 solids (VS), pH, water holding capacity (WHC) were performed in both labs, whilst the
151 elemental analysis (total carbon and total nitrogen contents) was performed in DUTH
152 only. Moisture content was measured through weight difference at 75ºC till constant
153 weight, whereas volatile solids were measured in a muffle furnace through the loss on
154 ignition of dried and ground material at 550ºC for 2 hours. Volatile solids, moisture and
155 pH were measured according to [31] and [32].Total carbon and total nitrogen were
156 measured using an elemental analyzer (CE Instruments, CHNS-O Model EA-1110),
157 according to [31] and [33].
158 Moisture, VS and pH measurements were done in 2 or up to 5 replications,
159 whereas total C and N were done in six replications. The replications aided to check the
160 statistical relationship between the initial parameters performed in UNIRC and DUTH
161 using ANOVA principles.
162 The WHC was measured according to method TS 11184 [34] by placing 1000 g

of sample in a previously weighed wet cotton bag, which was immersed in a water container for 24 h. The bag was removed from the water and allowed to drain for 6 h and then re-weighed. Table 2 shows which properties and respiration indices (discussed in next section) were measured in each laboratory.

In both labs, prior to the initiation of the dynamic respiration tests, all samples were optimized for moisture content by adding, if necessary, water to reach the 75% of the corresponding WHC [34]. In addition, bulk density was adjusted, where needed, by adding a styrofoam material to maintain a value for the mixture below 450-500 kg/m^3.

2.3. Dynamic respiration activity tests

2.3.1. Dynamic respiration index at UNIRC (Italy)

The dynamic respiration activity in UNIRC was measured using a 30 L adiabatic respirometric reactor (Costech International, Cernusco sulNaviglio, Italy) according to TS 11184 method [34] and [30]. The characteristic of the Costech® reactor is that it is adiabatic and that air flow entering the reactor is continuously adjusted so that to keep the O_2 concentration in the outlet stream between 14% and 17% (v/v), as suggested by [5-6, 25] and [4].

Advantages of the Costech® system include: i) the large volume of the reactor that can help minimizing sampling errors, especially for heterogeneous materials characterized by a relatively large size of the particles, ii) the automation of oxygen and temperature measurements, and iii) the ability for continuous automatic adjustment of the air flow. The main disadvantages are related to the high investment cost and the maintenance of the apparatus. Moreover, there is a need of controlling the temperature of the inlet air, especially in case of low biological activity, since the results of the test

are influenced by eventual significant changes in the temperature of the air supplied to the reactor. In terms of applicability, the main issue is the availability of the substrate, each time measured, because the operational quantity needed is -at least- 3.0 kg.

The hourly oxygen uptake rate (OUR_h) was calculated according to [30]:

$$OUR_h\ [mg\ O_2\ kg^{-1}\ VS\ h^{-1}] = Q * \Delta O_2 * V_g^{-1} * 31.98 * VS^{-1} \quad (1)$$

Where:

$Q\ [L\ h^{-1}]$ is the airflow rate

$\Delta O_2\ [mL\ L^{-1}]$ is the difference between the inlet and outlet O_2 concentrations of the reactor

$V_g\ [L\ mol^{-1}]$ is the volume of 1 mol of gas at the inlet air temperature

$31.98\ [g\ mol^{-1}]$ is the molecular weight of O_2

VS [kg] is the initial weight of volatile solids placed in the reactor.

Oxygen content measurements were performed every one hour. The DRI_{24} was then calculated as the moving average of the 24 OUR_h values, taken over the 24 h period that had the most intense biological activity. The amount of each substrate placed in the reactor ranged from 3.7 to 8.2 kg (wet weight) depending on bulk density (and availability of materials), whilst the volume occupied in the reactor was always maintained similar for all runs (approximately 15 L). The temperature in CosTech® reactors was measured via a probe placed in the centre of the biomass. One run was performed for each substrate (n=1), which is considered sufficient and representative due to the relatively large amount (> 3.5 kg) of the sample placed in the reactor [8].

2.3.2. Dynamic respiration index at DUTH (Greece)

The dynamic respiration activity in DUTH was measured in 5.5 L dynamic reactors made from plexi-glass, as described in detail by [24]. The air flowrate was adjusted with a volumetric flowmeter. Reactors were placed in a heated room at a constant temperature of 35±2^{o}C, but no temperature measurements within the reactor were performed. A wet sample of 500 g was placed in each reactor for each substrate. The unit air flow (UAF) rate for all eight substrates was maintained around 12 L air kg^{-1}VS h^{-1}; thus, the actual air flow rate was adjusted accordingly from 15 to 60 mL min^{-1}. In any case, the UAF rate never exceeded 140mL air dry kg^{-1} min^{-1} according to [35] suggestions.

Measurements of the instantaneous O_2 and CO_2 contents at the reactors' outlet were performed manually, using a portable gas analyzer (GA-2000P, Geotechnical Instruments Ltd., U.K). The frequency of the measurement varied, from three to eight times per day, depending on the biological activity of each substrate. The most intense biological activity, characterized by high O_2 consumption and high CO_2 generation, was observed during the first 4 days from the initiation of the experiments. In this initial period, more frequent measurements were made to better formulate the respiration activity profile. During the last days of the experiments, the measurement frequency was decreased, since O_2 consumption and CO_2 generation profiles tended to stabilize. The same sampling frequency had been performed and justified in [24].

The DRI_{24} was calculated similarly to above [4, 9]. The maximum CO_2 generation rate (D-CO_2_24) was calculated as the maximum of the averages of the instantaneous carbon dioxide generation rates recorded during the same 24-hour period of higher activity. DRI measurements were done in triplicates for six substrates (OFMSWC,

SIM_FW1, FORW, FAMN, AMNC and SIM_FW2) and in duplicates for substrates HC_1 and HC_2. All replicates were run concurrently.

2.3.3. Other dynamic respiration activity indices

Other dynamic respiration indices that were calculated on the basis of the same measurements done when calculating DRI_{24}, were: cumulative dynamic O_2 consumption after 4 and 7 days ($DCRI_4$ and $DCRI_7$, respectively); calculated as the integral below the OUR curve from day 0 to days 4 and 7, respectively. In DUTH, the maximum dynamic CO_2 generation rate (D-CO_2_24) at 24h and a dynamic cumulative CO_2 generation after 7 days (D-CO_2_7) were also calculated.

2.4. Biologic Methane Potential (BMP)

BMP tests were performed in UNIRC in duplicates under mesophilic conditions. The amount of substrate used was between 1.3 to 22.8 wet g for each vessel. Tests were performed using a WTW OxiTop® AN6 (Germany) apparatus that consisted of three sets of 6 glass bottles (1.1 L volume) placed in thermostatic cabinet at 35±0.5 °C and equipped with a manometric head that recorded pressure increases due to biogas production. Each bottle was continuously mixed by a magnetic stirrer. Biogas produced passed through a NaOH solution (3M), for CO_2 adsorption and methane production was then measured by an eudiometer (water displacement method). Anaerobic inocula were collected at three different periods from the second stage of an anaerobic digester that was working in mesophilic conditions and was fed with agro-wastes (cattle manure, chicken manure, agriculture residuals and other industrial waste coming from the transformation of agriculture products such as olive and citrus). Immediately after

sampling, inocula were sieved (<1mm) to remove large fibrous materials (e.g. straw) and were kept under endogenous anaerobic conditions at 35°C (mesophilic inocula) for a minimum of 7-10 days to reduce blank biogas production. The BMP of the substrate was calculated (in NmL CH_4 g^{-1} VS) by subtracting the methane production of the inoculum (i.e. blank biogas production) from the gross methane production. The characteristics of the inocula ranged from 21.0 to 59.0 g TS L^{-1} with a VS content from 55% to 73% (% TS). The substrate to inoculum ratio (in g VS/g VS) was always kept at around 0.4 to 0.5. The methane yield of the inocula ranged from 3% to 20% of the methane yield of the main substrates, except in the case of HC_1 for which the methane yield was comparable to that of the inoculum.

2.5. Static Respiration Index (SRI)

Static respiration indices were measured only in DUTH using a 1 L static solid phase respirometer equipped with WTW OxiTop-C® manometric heads, following the methodology described in [23] and [10]. 50 to 70 g of wet substrate were placed in each static respirometer and moisture content was adjusted to an optimal moisture content range between 50% to 60% ww. A 50 mL plastic beaker, used as an alkaline trap (containing a 2N KOH solution), was placed inside the respirometer in order to quantify the amount of C-CO_2 generated by the substrate. Respirometers were firmly closed and momentarily opened daily, so that the maximum pressure drop inside the vessels within two consecutive aerations would never exceed 170 hPa for any of the substrates. Based on the ideal gas law, this maximum pressure drop corresponded to a residual O_2 content in the vessel equal to 5% v/v, which still sustains aerobic conditions. These maximum pressure drops were observed after 24 h during the first couple of days. All

283 measurements were performed at 35^{o}C and lasted 7 d in an incubator at the absence of
284 light. Experiments were performed with 4 to 6 replicates per substrate.

285 The indices calculated for the static solid phase test in this study were: the
286 maximum O_2 consumption rate recorded over 12h and 24h periods of highest activity
287 (SRI_{12}, SRI_{24}, in mg O_2 kg^{-1} VS h^{-1}), the cumulative O_2 consumptions after 4 and 7 days
288 ($SCRI_4$, $SCRI_7$, in g O_2 kg^{-1} VS h^{-1}) and the CO_2 generation after 7 days (S-CO_2, in g C-
289 CO_2 kg^{-1} VS) as explained in [10].

290

291 *Insert Table 2*

292

293 **3. RESULTS AND DISCUSSION**

294 *3.1. Substrates' characterization*

295 The initial properties of the substrates are included in Table 3, where it is shown
296 that there was a very good agreement between the initial properties (moisture content,
297 volatile solids and pH) of all substrates from both labs; this ensured that respirometric
298 experiments on all materials started with identical initial properties in both countries.
299 WHCs for half of the studied substrates were almost the same (HC_1, FORW, AMNC,
300 SIM_FW2), while for the rest seemed to slightly differ. The as-received moisture
301 contents of the eight substrates ranged from 18% (ww) for the OFMSWC, to 83% (ww)
302 for the FAMN. Volatile solids (i.e. organic matter) ranged from a low 23% (dw) for the
303 OFMSWC, to 96-98% (dw) for the simulated food waste substrates (SIM_FW1 and
304 SIM_FW2). C/N ratios, as measured through elemental analysis, ranged from 8.2 to 40,
305 for FAMN and HC_2, respectively. pH values ranged from 5.1 (for SIM_FW2, which
306 had the higher organic matter content) to 9.5 for HC_1 (composted kitchen waste and

307 wood ashes with a VS at 42% dw). Most of the substrates had a pH between 6 and 8,
308 while manures (FAMN and AMNC) were alkaline.
309
310 *Insert Table 3*
311
312 *3.2. Oxygen uptake profiles in dynamic methods*
313 Table 4 presents the dynamic respiration indices and the BMP results. The profiles
314 of the oxygen uptake rates of the eight substrates, as measured by both laboratories, are
315 included in Figure 1. Note that the mean values are shown only in the case of the Greek
316 tests (for which there was replication),while the errors bars in the graphs show the
317 standard errors. Apparently, a very good replication (coefficient of variation was always
318 less than 5%) existed for the Greek dynamic respiration tests.
319
320 *Insert Table 4 and Figure 1*
321
322 According to Figure 1, there were quite similar OUR profiles between the ITA and
323 GRE dynamic tests for substrates HC_1, HC_2, SIM_FW1, FAMN and SIM_FW2. The
324 largest deviation among the OURs recorded between the two labs was observed for
325 OFMSWC; this can be also confirmed by looking at the corresponding dynamic indices
326 of Table 4. For this substrate, the Greek index was unexpectedly high, whilst the Italian
327 $DRI_{24}$was close to values suggested in the past by [36], who had used similar materials
328 and similar equipment. This contradiction with regard to OFMSWC is hard to explain
329 and could be attributed to an inherent variability and unpredictability of biological
330 experiments, or to a heterogeneity of the sample which was derived from MSW. It

might be also attributed to the fact that the temperature of OFMSWC in CosTech® remained similar to that of the ambient air (around 20 ºC) throughout the run, whilst artificial mesophilic temperatures (≈35ºC) were always maintained in the Greek method.

For FORW, a higher early peak was observed in the Greek method, although a rather stable and slightly fluctuating OUR was observed in the Italian method with a peak at the end of the run. This periodic fluctuating OUR profile in the case of the CosTech® runs was also obvious for substrates HC_1, FORW, FAMN, AMNC, SIM_FW2 and might be attributed to diurnal temperature variations during the experiment, since the air that was introduced into the reactor was at ambient room temperature. A similar disagreement seemed to exist in the case of FORW, in which the GRE method resulted in higher OURs compared to the ITA method. In AMNC too, the Greek method resulted in higher OURs compared to the Italian method; yet, this substrate was a rather stable one with some of the lowest OURs recorded in both methods. According to Figure 1, the OUR profiles in the Greek system were much smoother than the CosTech® OUR profiles, despite the fact that data recording was less infrequent in the former case.

In the CosTech® system, only 2 substrates (SIM_FW1 and SIM_FW2) reached temperatures up to 45ºC after 1.5 days which were then reduced and maintained to between 30ºC and 40ºC till the end of the process. This agrees with the fact that SIM_FW1 and SIM_FW2 had two of the highest respiration activities. On the other hand, FAMN, which also had a high respiration activity in Italy, never reached such mesophilic temperatures. This was caused due to a malfunction of the automatic airflow adjusting system after day 8. However, the DRI_{24} values were still calculated during the first 7 days, in which the maximum biological activity was anyway observed.

355

356 *3.3. Effect of temperature and air flow on respiration activity*

357 Figure 2 presents the temperatures of all 8 substrates measured only in the Italian
358 respiration method. The temperature in CosTech® reactors was measured via a probe
359 placed in the centre of the biomass. In the case of the Greek method, experiments were
360 performed in a 35±2^{o}C temperature-controlled room, whilst the reactors cannot be
361 considered strictly adiabatic. Since no temperature measurements were performed in the
362 case of the Greek reactors, no temperature profile are shown.

363 According to Figure 2, only SIM_FW1 and SIM_FW2 reached temperatures up to
364 45 ºC, yet none reached thermophilic temperatures. Both these substrates had two of the
365 highest respiration activities, as measured in both Italy and Greece. Yet, FAMN, which
366 also had a high respiration activity as measured in Italy, had, surprisingly, one of the
367 lowest temperatures among all substrates, which is difficult to explain. The internal
368 temperatures of all other substrates (i.e. except SIM_FW1 and SIM_FW2) remained at
369 ambient values between 20 to 25ºC.

370 As shown in Figure 1, maximum deviations for OUR profiles are observed for
371 OFMSWC and AMNC, which can also be confirmed through their dynamic indices
372 (Table 4). This can be attributed to the fact that in Italian reactor, these two substrates
373 did not exceed 25^{o}C. Adversely, Greek dynamic reactors were always in a temperature
374 range between 34 to 37 ^{o}C. The lack of high temperatures in the other 5 substrates might
375 be charged to the low respiration activity of those substrates (Table 4). Moreover,
376 difference in reactor scale is important since it might affect the temperature profile of
377 the material.

378

Insert Figure 2

When designing solid waste treatment plants, it is also necessary to know the amount of air required to maintain optimum conditions for the degradation of organic substrates. For this reason, Figure 3 was drawn in order to study the effect of the cumulative (total) air flow that passed through the CosTech® system after 7 days, on the respiration activity. In Figure 3, only data from the Italian reactor are shown, since the flowrate in that system was continuously adjusted throughout the process. On the contrary, in the Greek dynamic method, a constant UAF rate was applied to all reactors throughout the experiments.

Insert Figure 3

According to Figure 3, it appears that as the total flow of air increases, the respiration activity increases too; yet it seems that a constant DRI_{24} value tends to be reached at high cumulative flows. This trend was also shown by [37] and can be explained by the fact that beyond a certain cumulative aeration threshold value, the respiration activity is maximized and stabilizes and no more excess air is necessary. Moreover, an excessive aeration could decrease microbial activity due to the reduction of the temperature of the biomass at sub-optimal levels.

3.4. Static and dynamic respiration indices

Table 5 includes the static respiration indices and the ratios of the dynamic respiration (measured in the Greek lab) over the static respiration indices. As shown in

Table 5, the dynamic respiration indices were in general much higher than the corresponding static indices (both in terms of rate and total oxygen consumption). No consistency was also observed between the dynamic and the static test indices in the recent work of Aspray et al. [20]. After categorizing the 8 substrates in fresh (SIM_FW1, SIM_FW2, FAMN) and stabilized composts (HC_1, HC_2, OFMSWC, FORW, AMNC), it was observed that the ratio of DRI_{24}/SRI_{24}(measured in Greece) for the fresh substrates ranged from 3.3 to 11.4 as opposed to the stable substrates for which that ratio ranged from only 0.9 to 3.3. Similarly, the ratio of $DCRI_7/SCRI_7$ ranged from 18 to 40, for the fresh substrates and from 1.2 to 13, for the stable substrates. The above indicates that the static methods underestimate actual oxygen consumption, as had been also suggested by [4]. This variability in the ratios of DRI to SRI contradicts the findings of [4], who showed that the dynamic respiration rate indices (DRI_{24}) of severalmunicipal solid waste derived organic substrates (fresh, composted and stable) were, on average, 2 times higher than the corresponding static respiration indices (SRI_{24}). It is noted, however, that [4] had measured oxygen consumption in their static methods with an oxygen probe at 20 ºC. Comparison with dynamic and static test were also conducted in the work of Godley et al. [28], who had also reported that dynamic respiration rates were at least double than the static ones. Similar results had been found by Adani et al. [6] in which all dynamic respiration rates were far higher compared to the static ones, based on a sample size of 18 organic substrates.

Insert Table 5

3.5. Correlations

Potential correlations among the initial physicochemical properties measured (OM content, pH, total carbon and total nitrogen) and the main biodegradation indices were calculated. The only positive correlation existed between total nitrogen content and the DRI_{24} and CH_4 production after 30 d (in both cases Spearman's ρ=0.8), as these were measured by the Italian lab. With regard to the aerobic index, this correlation can be explained by the nitrification processes that consume oxygen. On the other hand, neither total carbon nor organic matter contents correlated significantly with any of the main biodegradability indices, as also revealed in Figure 4. According to Figure 4, a clear outlier exists (OFMSWC), whilst it is observed that the substrates with VS contents above 85% (db) achieved a very wide range of respiration activities. As opposed to OFMSWC, FORW had a rather high VS content (around 80% dw) which would be expected to result to a relatively high respiration activity. However, this was not true, and the dynamic respiration indices (from both labs) were relatively low for that substrate (i.e. <500 mg O_2 kg^{-1} VS h^{-1}). This can be attributed to the likely low content of readily biodegradable substrate present in the leaves. Their high volatile solids content is most probably attributable to the high content of lignin, which is recalcitrant to fast biodegradation in aerobic conditions. From the above it appears that no clear relationship exists between the VS content of the materials and the respiration activity. Similar observations have been found out by Barrena et al. [8], in which the organic matter content did not correlate sufficiently with any of the dynamic or static respiration indices. On the contrary, Aspray et al. [20] found that the VS content correlated positively with two different dynamic methods, while no correlation was evident with the static test indices. Positive correlation between organic matter content and both O_2 and CO_2 static indices have been reported in [23] and [10]. Apparently, specific

constituents of the organic matter, such as sugar content (i.e. cellulose, hemicelluloses, lignin, starch), as well as the level of microbial inoculation, determine the biodegradation extents (and thus the respiration activity) regardless of total VS content.

Insert Figure 4

Figure 5 graphically depicts the most important correlations among the biodegradation indices measured in this work. Table 6 includes all Spearman's rank order correlation coefficients among the studied indices (only the statistically significant values at $p<0.05$ are shown). Note that the Spearman coefficients calculate any type of correlation and not necessarily a linear one (as in the case of the Pearson coefficients).

Include Figure 5 and Table 6.

According to Table 6, the DRI_{24} indices from both labs correlated between them adequately ($\rho=0.74$) with a marginally statistically significant correlation at $p<0.05$. According to Figure 5a, OFMSWC appears to be an extreme value. Similarly to DRI_{24}, the cumulative oxygen consumptions ($DCRI_4$, $DCRI_7$) also correlated significantly in both labs (coefficients ranged from 0.83 to 0.95), verifying the visible positive correlation trend illustrated in Figure 5b. According to Table 6, the cumulative dynamic respiration indices ($DCRI_4$, $DCRI_7$) also correlated well with the corresponding DRI_{24} in both labs. This was also shown in [24] and practically indicates that the higher the maximum O_2 consumption rate, the higher the total oxygen consumption too.

Table 6 also reveals a very strong correlation between the methane generation

after 30 days and the dynamic respiration indices of both labs. This is also evident in Figures 5c, 5d that both illustrate that positive correlation between both Greek and Italian dynamic respiration indices (DRI_{24}) and the 30-d methane yield. This is an important finding, because it indicates that the degradation potential of an organic substrate could be similar under both aerobic and anaerobic environments, as has been shown by other researchers too [8, 15, 38]. However the values of the dynamic respiration indices measured, respectively, in the Italian laboratory, for OFMSWC, and in the Greek laboratory, for SIMFW_2, worsened significantly this correlation.

According to Figures 5e and 5f, the correlation among the static and dynamic respiration indices (DRI_{24} and SRI_{24}) was poor as was discussed in section 3.4 too.

The correlation between the total CO_2 generation and the total oxygen consumption under dynamic conditions measured in the Greek lab was strong (correlation coefficients above 0.98), as shown in Table 6. This indicates that the moles of generated CO_2 were steadily proportional to the moles of O_2 consumed for all substrates. This was also noticed for the peak rates of CO_2 generation (D-CO_2_24) and the peak rates of O_2 consumption (DRI_{24}) indicating that CO_2 generation rate and OUR followed a parallel profile (in the dynamic tests in Greece). Moreover, respiratory quotients (RQ: moles of CO_2 generated over the moles of O_2 consumed) calculated for the dynamic experiments conducted in Greece, ranged between 0.7 and 1.0 (Table 4), which are reasonable values and comparable to [24].

Statistically significant correlation was also observed between the static CO_2 generation and the static O_2 consumption values obtained in the Greek lab (ρ=0.91). Therefore, CO_2 generation could be also used as a biodegradation activity index, although a caution is required, since static indices are affected by temperature and CO_2

evolution is pH-dependent due to its solubility in aqueous solutions [12]. In the case of static method RQs ranged from 1.3 to 2.3, with an average value of 1.7 (Table 5). High RQ values -using the same static method- had been also reported by Komilis et al. [10] indicating variations in the oxidation stage and the degradability of the carbon contained in each substrate.

3.6. Use of biodegradation activity indices for compliance purposes

According to the findings of this research, the use of both aerobic dynamic respiration and anaerobic methods is an important tool to evaluate the biological activity of fresh and composted biodegradable wastes/residues. With regard to fresh substrates, this is important knowledge for the selection of the appropriate treatment technologies. For practical purposes, and by accounting for the good correlation of the aerobic and anaerobic indices, the use of the former indices would be preferred (shorter duration than the anaerobic ones, no need of seed). Moreover, the contemporary use of aerobic and anaerobic methods allows a cross check and the detection of errors or of measurement problems (e.g. inhibition) that are frequent with this type of complicated biological measurements. This is very important when for practical reasons, as in the Di.Pro.Ve-CosTech® system, replicates are difficult to perform. Therefore, the biological stability index presented in this paper, and especially the DRI_{24} and BMP, could be adopted for process control at the level of single plant. In this case, when the indices are used only for internal purposes, the method and equipment to be adopted for the measurements could be chosen based on the cost of the equipment and on the knowledge of the plant's technical personnel.

However, the use of stability indices for compliance purposes (e.g. evaluation of

compost quality, acceptance of waste in landfill) is more complicated. In fact, compliance threshold values must be referred to a specific method as in norms in Germany and Italy [26-27] and should be set considering the uncertainty connected to this type of measurements that is higher when replicates are not provided. In this case, also, the adoption of a double compliance (both to aerobic and anaerobic threshold values) could help to ensure the reliability of the evaluation and the detection of errors/measurement problems as done in Germany and Austria. However, practical problems linked to the long duration of the tests (at least 7 days for DRI and 30 days for BMP) should be solved prior to a generalized adoption of compliance tests based on biological activity methods.

A reasonable possibility would be to measure the stability indices (both dynamic aerobic and anaerobic) with a frequency related to the amount of biomass treated in the plant; the evaluation of the compliance should then be done on a statistical basis (e.g. 95% of the samples analysed yearly should comply with the threshold values). If the frequency of samples with respiration indices above the threshold imposed by the norms exceeds a pre-set value, some form of penalty should be provided.

In the case of using indices for compliance purposes, the adoption of standard and unified methods among countries, at least at a European Level, would be greatly beneficial.

4. CONCLUSIONS

Results of the work indicate that although an agreement existed among the different methods for some of the substrates, differences did exist for others so that there is no unique ideal method to propose. In addition, the OUR profile was much

547 smoother in the case of the Greek dynamic reactor compared to the CosTech® system,
548 despite the smaller recording interval in the former case. A statistically significant
549 correlation was calculated between the dynamic respiration indices in both labs, with
550 regard to both rates and cumulative values. However, a weak agreement was observed
551 for one of the substrates. A strong correlation was found between the methane yield
552 after 30 days and the 24-h based dynamic respiration rate and the cumulative dynamic
553 respiration indices, at 4 and 7 days. The ratio of the dynamic to static respiration rates
554 ranged from 1 to 11 and was higher in fresh materials compared to the more stable ones.
555 The dynamic respiratory quotients were very close to 1.0, whilst the static ones had an
556 average of 1.7. Finally, it appears that the volatile solids content should not be used as a
557 predictor of biodegradation activity.
558
559 **REFERENCES**
560 1. Iannotti, D.A., Pang, T., Toth, B.L., Elwell, D.L., Keener, H.M. & Hoitink, H.A.J.: A
561 quantitative respirometric method for monitoring compost stability. Compost Sci.
562 Util. 1, 52-65 (1993).
563 2. Lasaridi, K, Stentiford, E.: A simple respirometric technique for assessing compost
564 stability, Water Res. 32 (12), 3717-3723 (1998).
565 3. Binner, E., Zach, A.: Laboratory tests describing the biological reactivity of
566 pretreated residual wastes. in Proc. of the ORBIT symposium, Weimar, Germany
567 (1999).
568 4. Scaglia, B., Tambone, F., Genevini, P.L., Adani, F.: Respiration index determination:
569 A dynamic and static approach. Compost Sci. Util. 8, 90-98 (2000).
570 5. Adani, F., Lozzi, P., Genevini, P.L.: Determination of biological stability by oxygen

571 uptake on municipal solid waste and derived products. Compost Sci. Util. 9, 163-178
572 (2001).
573 6. Adani, F., Gigliotti, G., Valentini, F., Laraia, R.: Respiration index determination: a
574 comparative study of different methods. Compost Sci. Util. 13, 144-151 (2003).
575 7. Tremier A., de Guardia, A., Massiani, C., Paul, E., Martel, J.L.: A respirometric
576 method for characterising the organic composition and biodegradation kinetics and
577 the temperature influence on the biodegradation kinetics, for a mixture of sludge and
578 bulking agent to be co-composted. Bioresource Technol. 96, 169-180 (2005).
579 8. Barrena, R., d'Imporzano, G., Ponsá, S., Gea, T., Artola, A., Vasquez, F., Sánchez,
580 A., Adani, F.: In search of a reliable technique for the determination of the biological
581 stability of the organic matter in the mechanical-biological treated waste. J. Hazard.
582 Mater. 162, 1065-1072 (2009).
583 9. Ponsá, S., Gea, T., Sánchez, A.: Different indices to express biodegradability in
584 organic solid wastes. J. Environ. Qual. 39, 706-712 (2010b).
585 10. Komilis, D., Kontou, I., Ntougias, S.: A modified static respiration assay and its
586 relationship with an enzymatic test to assess compost stability and maturity.
587 Bioresource Technol. 102, 5863-5872 (2011).
588 11. Oviedo-Ocaña, E.R., Torres-Lozada, P., Marmolejo-Rebellon, L.F., Hoyos, L.V.,
589 Gonzales,S., Barrena, R., Komilis, D., Sanchez, A.: Stability and maturity of
590 biowaste composts derived by small municipalities: Correlation among physical,
591 chemical and biological indices. Waste Manage. 44, 63-71 (2015).
592 12. Barrena, R., Vázquez, F., Sánchez, A.: The use of respiration indices in the
593 composting process: a review. Waste Manage. Res. 24, 37-41 (2006).
594 13. Baffi, C., Dell'Abate, M.T., Nassisi, A., Silva, S., Benedetti, A., Genevini, P.L.,

595 Adani, F.: Determination of biological stability in compost: A comparison of
596 methodologies. Soil Biol. Biochem. 39, 1284-1293 (2007).
597 14. Adani, F., Confalonieri, R., Tambone, F.: Dynamic respiration index as a descriptor
598 of the biological stability of organic wastes. J. Environ. Qual. 33(5), 1866-1876
599 (2004).
600 15. Ponsá, S., Gea, T., Alerm, L., Cerezo, J., Sánchez, A.: Comparison of aerobic and
601 anaerobic stability indices through a MSW biological treatment process. Waste
602 Manage. 28, 2735-2742 (2008).
603 16. Schievano, A., Pognani, M., D'Imporzano, G., Adani, F.: Predicting anaerobic
604 biogasification potential of ingestates and digestates of a full-scale biogas plant using
605 chemical and biological parameters. Bioresource Technol. 99, 8112-8117 (2008).
606 17. Pognani, M., Barrena, R., Font, X., Scaglia, B., Adani, F., Sánchez, A.: Monitoring
607 the organic matter properties in a combined anaerobic/aerobic full-scale municipal
608 source-separated waste treatment plant. Bioresour. Technol. 101, 6873-6877 (2010).
609 18. Brinton, W.F., Evans, E., Droffner, M.L., Brinton, R.B.: A standardized Dewar test
610 for evaluation of compost self-heating. http://woodsend.org/pdf-files/dewar_re.pdf
611 (1995). Accessed 16 February 2016.
612 19. Koening, A., Bari, Q.H.: Application of self-heating test for indirect estimation of
613 respirometric activity of compost: theory and practice. Compost Sci. Util. 8, 99-107
614 (2000).
615 20. Aspray, T.J., Dimambro, M.E., Wallace, P., Howell, G., Frederickson, J.: Static,
616 dynamic and inoculum augmented respiration based test assessment for determining
617 in-vessel compost stability. Waste Manage. 42, 3-9 (2015).
618 21. Ponsá, S., Gea, T., Sánchez, A.: The effect of storage and mechanical pretreatment

619 on the biological stability of municipal solid wastes. Waste Manage. 30, 441-445
620 (2010a).
621 22. Gea, T., Barrena, R., Artola, A., Sánchez, A.: Monitoring the biological activity of
622 the composting process: oxygen uptake rate (OUR), respirometric index (RI) and
623 respiratory quotient (RQ). Biotechnol. Bioeng. 88, 520-527 (2004).
624 23. Komilis, D., Tziouvaras, I.: A statistical analysis to assess the maturity and stability
625 of six composts. Waste Manage. 29, 1504-1513 (2009).
626 24. Komilis, D., Kanellos, D.: A modified dynamic respiration test to assess compost
627 stability: Effect of sample size and air flowrate. Bioresource Technol. 117, 300-309
628 (2012).
629 25. Adani, F., Ubbiali, C., Generini, P.: The determination of biological stability of
630 composts using the dynamic respiration index: the results of experience after two
631 years. Waste Manage. 26, 41-48 (2006).
632 26. Federal Ministry for the Environment: Nature Conservation and Nuclear Safety
633 Ordinance on Environmentally Compatible Storage of Waste from Human
634 Settlements of 20 February 2001. Federal Law Gazette, Germany, p.305 (2001).
635 27. Decree of the Veneto Region Government: Putrescibilità dei rifiuti: definizione e
636 determinazione analitica. [Putrescibility of refuse: definitions and analytical
637 determination] Allegato A al DGR n. 2254 del 08.08.2008 (2008).
638 28. Godley, A.R., Graham, A., Lewin, K: Estimating biodegradable municipal solid
639 waste diversion from landfill: screening exercise to evaluate the performance of
640 biodegradable test methods, R&D Technical Report P1-513 (EP0173) phase 1,
641 United Kingdom Environment Agency (2005).
642 29. European Committee for Standardization: Solid recovered fuels – Determination of

643 the current rate of aerobic microbial activity using the real dynamic respiration index.
644 EN 15590:2011 (2011).
645 30. Scaglia, B., Acutis, M., Adani, F.: Precision determination for the dynamic
646 respirometric index (DRI) method used for biological stability evaluation on
647 municipal solid waste and derived products. Waste Manage. 31, 2-9 (2011).
648 31. U.S. Department of Agriculture (USDA), U.S. Composting Council (USCC): In:
649 Thomson, W. (Ed.), Test Methods for the Examination of Composting and Compost,
650 The Composting Council Research and Education Foundation, Holbrook, New York,
651 03.09.1-03.09.4 (2002).
652 32. APHA, AWWA: Standard Methods for the Examination of Water and Wastewater.
653 20th ed. Washington, DC: American Public Health Association (1998).
654 33. Komilis, D., Evangelou, A., Giannakis, G., Lymperis, C.: Revisiting the elemental
655 composition and the calorific value of the organic fraction of municipal solid wastes.
656 Waste Manage. 32, 372-381 (2012).
657 34. UNI (Italian Organization for Standardization): Potential Dynamic Respiration
658 Index (PDRI) Determined Using Method UNI/TS 11184:2006 (Waste and Refuse
659 Derived Fuels - Determination of Biological Stability by Dynamic Respirometric
660 Index) UNI, Milan, IT (in Italian) (2006).
661 35. ASTM: Standard Test Method for Determining the Stability of Compost by
662 Measuring Oxygen Consumption (re-approved 2004). American Society for Testing
663 and Materials, D 5975-96 (1996).
664 36. Scaglia, B., Adani, F.: An index for quantifying the aerobic reactivity of municipal.
665 Sci. Total Environ. 394, 183-191 (2008).

37. Almeira, N., Komilis, D., Barrena, R., Gea, T., Sanchez, A.: The importance of aeration mode and flowrate in the determination of the biological activity and stability of organic wastes by respiration indices. Bioresource Technol. 196, 256-262 (2015).

38. Cossu, R., Raga, R.: Test methods for assessing the biological stability of biodegradable waste. Waste Manage. 28, 381-388 (2008).

TABLES

Table 1. Characteristics of the organic substrates

Substrate	Type	Source materials	Composting period	Curing or storage time
SIM_FW1	Raw food waste (simulated)	Apples, cooked meat, boiled pasta, bread (25% each, in wb)	-	1 week
SIM_FW2	Raw food waste (simulated)	Uncooked pasta, uncooked fries, beef based dog food (50%, 30%, 20%, in wb respectively)	-	1 week
FAMN	Fresh animal manure	Cow manure and straw	-	1 week
HC_1	Home compost	Mix of kitchen and garden waste and ash	6months	6months
HC_2	Home compost	Mix of kitchen waste and wood chips	3 weeks forced aeration	2 weeks
FORW	Forest waste	Leaves and small branches from forest floor	-	-
AMNC	Animal manure derived compost	Cow manure and straw	n/a	1 week after solid-liquid separator
OFMSWC	Compost from the organic fraction of MSW	Organic fraction of municipal solid wastes	Aeration for 28 days in a MBT plant	n/a

n/a: no available info

Table 2.Parameters and biodegradation activity indices measured in both laboratories

	Greek lab (DUTH)	Italian lab (UNIRC)	References
Moisture	✓	✓	USDA and USCC (2002);APHA and AWWA (1998)
Volatile solids	✓	✓	
pH	✓	✓	
WHC	✓	✓	TS 11184, UNI (2006)
Total carbon and nitrogen	✓	×	USDA and USCC (2002); Komilis et al (2012)
Dynamic method			
O_2 consumption rate at 24 hr (DRI_{24})	✓	✓	Scaglia et al. (2000); Adani et al. (2001); TS 11184, UNI (2006); Ponsa et al. (2010b)
Cumulative O_2 consumption after 4 d ($DCRI_4$)	✓	✓	
Cumulative O_2 consumption after 7 d ($DCRI_7$)	✓	✓	
CO_2 generation rate at 24 hr (D-CO_2_24)	✓	×	
Cumulative CO_2 generation after 7 d (D-CO_2_7)	✓	×	Komilis and Kanellos (2012)
Respiratory Quotient (RQ)	✓	×	
Biologic Methane Potential			
CH_4 generation after 30 d (BMP_{30})	×	✓	Schievano et al. (2008); Calabro et al. (2015)
Static method			
O_2 consumption rate at 12 hr (SRI_{12})	✓	×	
O_2 consumption rate at 24 hr (SRI_{24})	✓	×	
Cumulative O_2 consumption after 4 d ($SCRI_4$)	✓	×	Komilis et al. (2011)
Cumulative O_2 consumption after 7 d ($SCRI_7$)	✓	×	
Cumulative CO_2 generation after 7 d (S-CO_2)	✓	×	
Respiratory Quotient (RQ)	✓	×	

Table 3. Initial properties of the substrates used in the experiments[*]

Substrate	Moisture (% wb)		Volatile Solids (% db)		pH		WHC (g H_2O/wet kg)		C/N
	GRE (n=3)	**ITA** (n=1)	**GRE** (n=5)	**ITA** (n=4)	**GRE** (n=2)	**ITA** (n=1)	**GRE** (n=1)	**ITA** (n=1)	**GRE** (n=6)
SIM_FW1	61%±1.0%	60%	96%±0.3%^{B}	97%±0.0%^{A}	5.4±0.0	5.0	729	938	13.8±0.6
SIM_FW2	40%±2.0%^{A}	39%$^{+}$±1.1%^{A}	98%±0.0%^{B}	95%±0.8%^{A}	5.1±0.0	5.5	561	565	19.6±0.4
FAMN	79%±0.1%	83%	93%±0.3%^{B}	92%±0.1%^{A}	8.3±0.1	7.5	969	1200	8.2±0.1
HC_1	66%$^{+}$±0.4%	68%	42%±0.3%^{A}	41%±1.3%^{A}	8.8	9.5	798	809	24.6±1.6
HC_2	62%±0.8%	59%	93%±0.1%^{B}	94%±0.2%^{A}	7.2±0.1	8.2	1241	1000	39.5±2.1
FORW	74%±0.2%	71%	81%±0.4%^{A}	82%±2.2%^{A}	6.2±0.1	6.9	841	861	33.9±1.3
AMNC	40%±0.5%^{A}	41%$^{\wedge}$±0.7%^{A}	32%±0.2%^{A}	32%±0.8%^{A}	8.1±0.0	8.4	814	831	12.1±0.1
OFMSWC	22%±1.4%	18%	23%±2.0%^{A}	26%±1.2%^{A}	7.7±0.1	8.1	633	456	21.9±2.1

[*]: all values are averages ± standard errors; $^{+}$: n=2; $^{\wedge}$: n=4; ww: wet weight basis; dw: dry weight basis; WHC: water holding capacity. Means, per parameter and substrate in the same row, that do not share a letter, are significantly different based on Tukey's test (at $p<0.05$); (for example, the VS contents of HC_1 are statistically similar between the 2 labs, but statistically different for HC_2).

Table 4.Results of the dynamic respiration and BMP tests*

Substrate	DRI_{24} (mg O_2 kg^{-1} VSh^{-1})		$DCRI_4$ (g O_2 kg^{-1} VS)		$DCRI_7$ (g O_2 kg^{-1} VS)		D-CO_2_24 (mg C kg^{-1} VS h^{-1})	D-CO_2_7 (g C kg^{-1} VS)	RQ	BMP_{30} (NL CH_4 kg^{-1} VS)
	GRE	ITA[1]	GRE	ITA[1]	GRE	ITA[1]	GRE	GRE	GRE	ITA
SIM_FW1	2721[3]±111	3522	102[3]±9.6	225.4	292[3]±18.1	446.0	1010[3]±51	105[3]±12.2	1.0[3]±0.0	467[1]
SIM_FW2	2921[3]±26	1902	186[3]±0.0	147.2	327[3]±11.2	247.4	1068[3]±16	106[3]±4.0	1.0[3]±0.0	246[1]
FAMN	2327[3]±40	2369	192[3]±6.7	143.6	345[3]±8.9	174.1	1585[3]±29	218[3]±6.2	1.8[3]±0.0	352±6.5[2]
HC_1	255[2]±43	250	20.3[2]±3.8	16.8	37.6[2]±6.2	32.9	73[2]±15	10.8[2]±1.4	0.8[2]±0.1	55±8.1[2]
HC_2	1259[2]±6	949	99.7[2]±0.3	71.4	155[2]±0.9	98.3	350[2]±1	44.4[2]±0.1	0.7[2]±0.0	168[1]
FORW	474[3]±8	245	35.8[3]±0.3	20.2	57.1[3]±0.6	33.7	159[3]±27	17.9[3]±3.1	0.9[3]±0.2	167±11.4[2]
AMNC	696[3]±71	148	45.5[3]±1.7	8.5	88.1[3]±3.6	17.6	183[3]±27	20.9[3]±1.5	0.7[3]±0.0	163±26.2[2]
OFMSWC	2385[3]±25	371	182[3]±2.3	30.4	282[3]±5.1	48.1	682[3]±13	82.9[3]±1.8	0.8[3]±0.0	291±6.6[2]

*: all values are averages ± standard errors; [1]: n=1; [2]: n=2, [3]:n=3; DRI_{24}: 24-h based dynamic respiration index; $DCRI_4$: dynamic cumulative respiration index at 4 days; $DCRI_7$: dynamic cumulative respiration index at 7 days; D-CO_2_24: 24-h based dynamic CO_2 production rate; D-CO_2_7: dynamic CO_2 cumulative generation at 7 days; CH_4_30: methane generation after 30 days based on the BMP tests. GRE: Greece, ITA: Italy.

Table 5. Results of the static respiration indices and respiration ratios for the Greek lab*

Substrate[1]	SRI_{24} (mg $O_2 kg^{-1}$ VSh^{-1})	$SCRI_4$ (g $O_2 kg^{-1}$ VS)	$SCRI_7$ (g $O_2 kg^{-1}$ VS)	C-CO_2 (g C kg^{-1} VS)	RQ	DRI_{24} / SRI_{24}	$DCRI_7$ / $SCRI_7$
SIM_FW1	330±12	10.6±0.3	11.8±0.4	10.3±0.7	2.3±0.1	8.2	24.7
SIM_FW2	256±13	7.3±0.4	8.2±0.4	5.6±0.2	1.8±0.0	11.4	39.9
FAMN	705±32	18.2±0.9	19.3±0.9	11.3±0.3	1.6±0.1	3.3	17.9
HC_1	273±12	20.6±1.1	31.3±1.0	15.5±0.7	1.3±0.0	0.9	1.2
HC_2	434±19	11.1±0.4	11.7±0.4	6.1±0.1	1.4±0.1	2.9	13.3
FORW	400±29	20.5±1.0	22.6±0.9	10.9±0.2	1.3±0.0	1.2	2.5
AMNC	208±17	13.8±2.0	19.4±0.8	11.5±1.0	1.6±0.1	3.3	4.5
OFMSWC	759±69	23.8±0.8	25.5±0.9	20.8±0.1	2.2±0.1	3.1	11.0

*: all values are averages ± standard errors;[1]: n=4 for substrates HC_1, OFMSWC, AMNC; n=5 for substrates HC_2, SIM_FW2; n=6 for substrates SIM_FW1, FORW, FAMN; SRI_{24}: 24-h based static respiration index; $SCRI_4$: static cumulative respiration index at 4 days; $SCRI_7$: static cumulative respiration index at 7 days; C-CO_2: static cumulative CO_2 generation at 7 days;

Table 6. Spearman rank-order correlation coefficients among various indices

	VS	DRI_{24} [G]	$DCRI_4$ [G]	$DCRI_7$ [G]	D-CO_2_24 [G]	D-CO_2_7 [G]	SRI_{12} [G]	SRI_{24} [G]	$SCRI_4$ [G]	$SCRI_7$ [G]	S-CO_2_7 [G]	DRI_{24} [I]	$DCRI_4$ [I]	$DCRI_7$ [I]
DRI_{24}[G]	n/s													
$DCRI_4$[G]	n/s	0.83												
$DCRI_7$[G]	n/s	0.86	0.98											
D-CO_2_24[G]	n/s	0.86	0.98	1.00										
D-CO_2_7[G]	n/s	0.86	0.98	1.00	1.00									
SRI_{12}[G]	n/s	n/s	0.76	0.71	0.71	0.71								
SRI_{24}[G]	n/s	n/s	n/s	n/s	n/s	n/s	0.86							
$SCRI_4$[G]	-0.83	n/s	n/s	n/s	n/s	n/s	n/s	n/s						
$SCRI_7$[G]	-0.81	n/s	n/s	n/s	n/s	n/s	n/s	n/s	0.93					
S-CO_2_7[G]	-0.88	n/s	n/s	n/s	n/s	n/s	n/s	n/s	0.88	0.91				
DRI_{24}[I]	0.76	0.74	0.71	0.81	0.81	0.81	n/s	n/s	n/s	n/s	n/s			
$DCRI_4$[I]	0.83	0.83	0.71	0.81	0.81	0.81	n/s	n/s	n/s	-0.71	n/s	0.95		
$DCRI_7$[I]	0.83	0.83	0.71	0.81	0.81	0.81	0.62	n/s	n/s	-0.71	n/s	0.95	1.00	
CH_4_30[I]	n/s	0.83	0.83	0.88	0.88	0.88	0.74	n/s	n/s	n/s	n/s	0.83	0.81	0.81

All Spearman's rank-order correlation coefficients shown are significant at $p<0.05$; n/s: non-significant;
[G]: Greek method; [I]: Italian method.

Figure captions

Figure 1.Oxygen uptake rates (OUR) for the studied substrates using the dynamic respiration activity methods in Greece (GRE) and Italy (ITA).

Figure 2. Temperature profiles of the studied substrates, in the CosTech® dynamic reactors (Italian method).

Figure 3. Effect of cumulative air flow on respiration activity, in the CosTech® dynamic reactors (Italian method).

Figure 4.Correlation plots between the initial volatile solids content of the substrates and: (a) DRI_{24}, (b) $DCRI_{7}$ measured in the Greek (GRE) and the Italian (ITA) labs.

Figure 5. Correlation plots between Greek (GRE) and Italian (ITA) indices; (a) and (b): dynamic indices;(c) and (d): BMP and dynamic indices; (e), (f): static and dynamic indices.

Figure 1

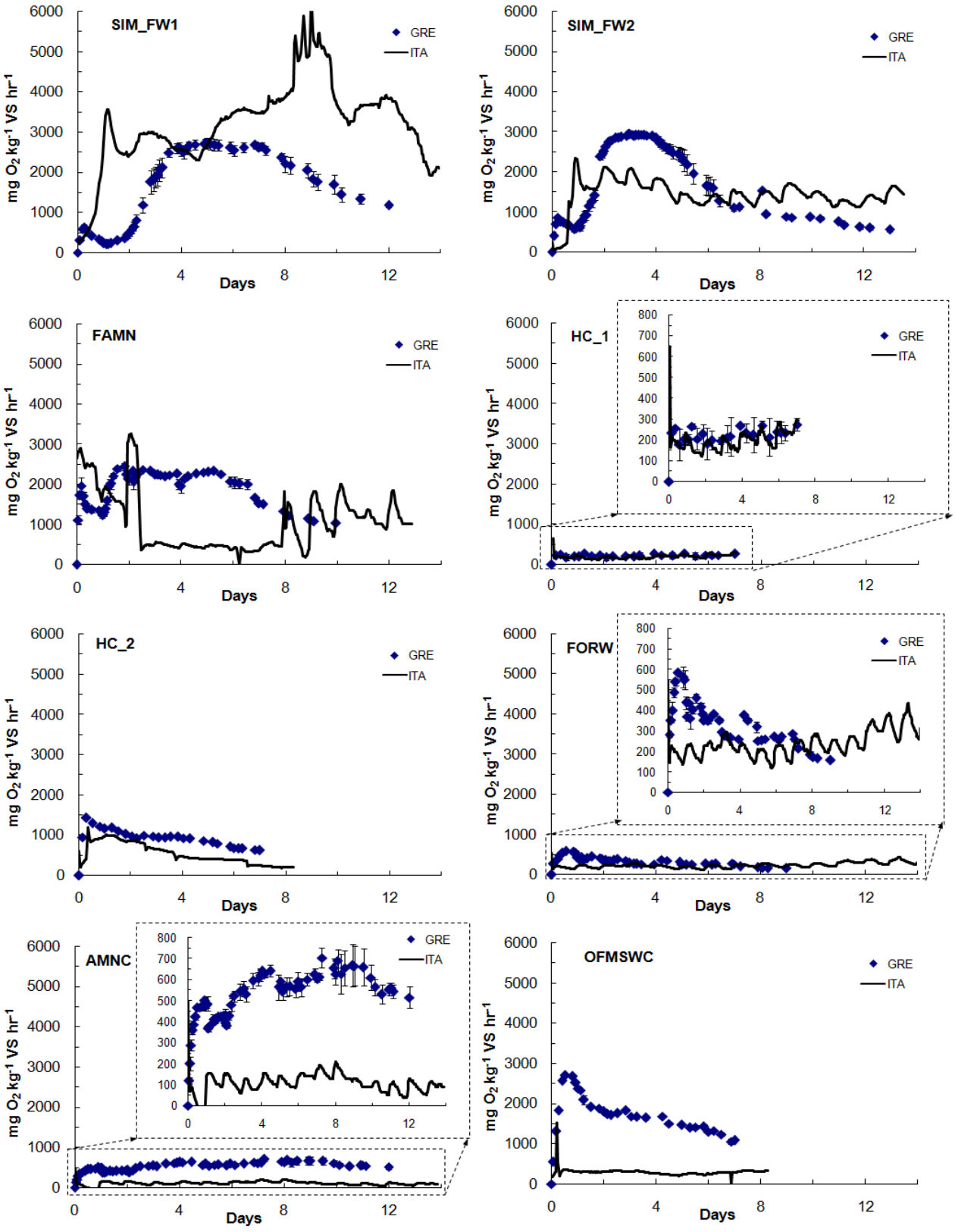

SIM_FW1
SIM_FW2
FAMN
HC_1
HC_2
FORW
AMNC
OFMSWC
GRE
ITA
mg O2 kg-1 VS hr-1
Days

Figure 2

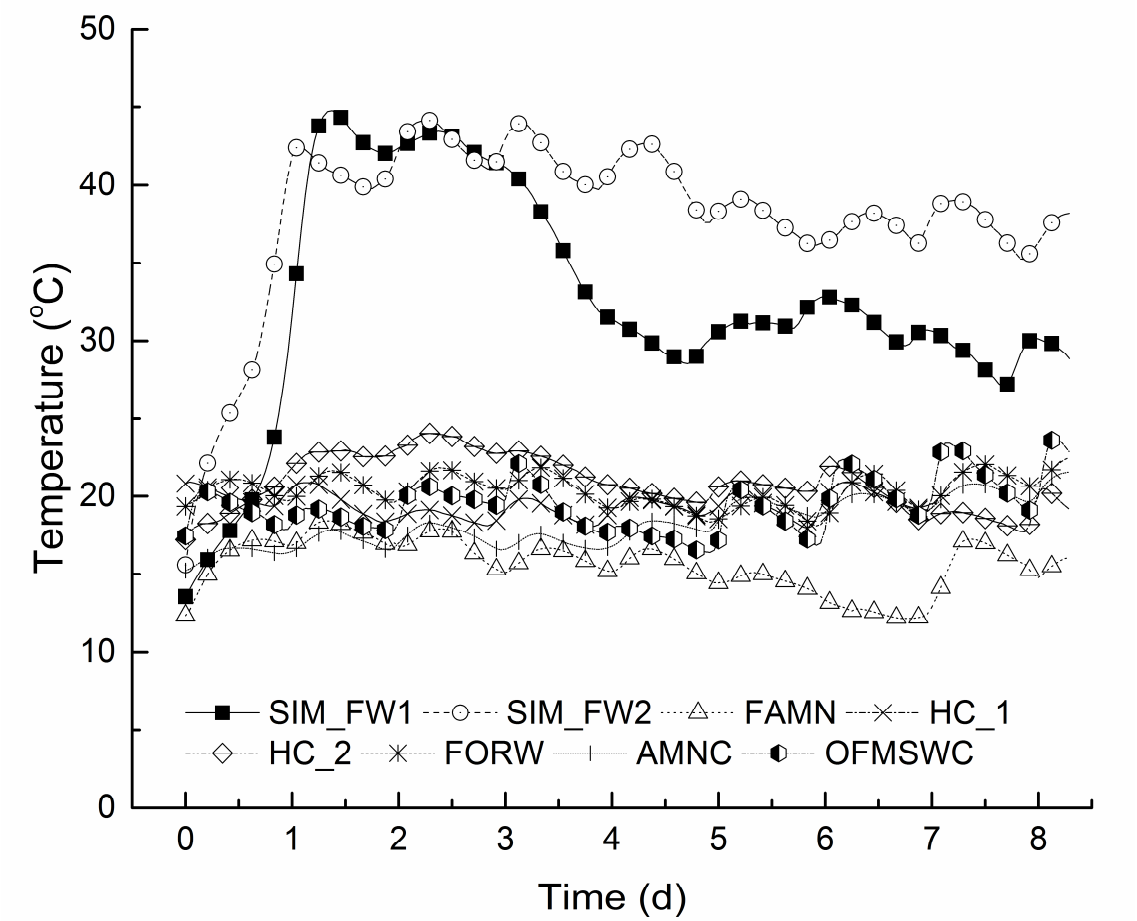

Temperature (°C)
Time (d)
SIM_FW1
SIM_FW2
FAMN
HC_1
HC_2
FORW
AMNC
OFMSWC

Figure 3

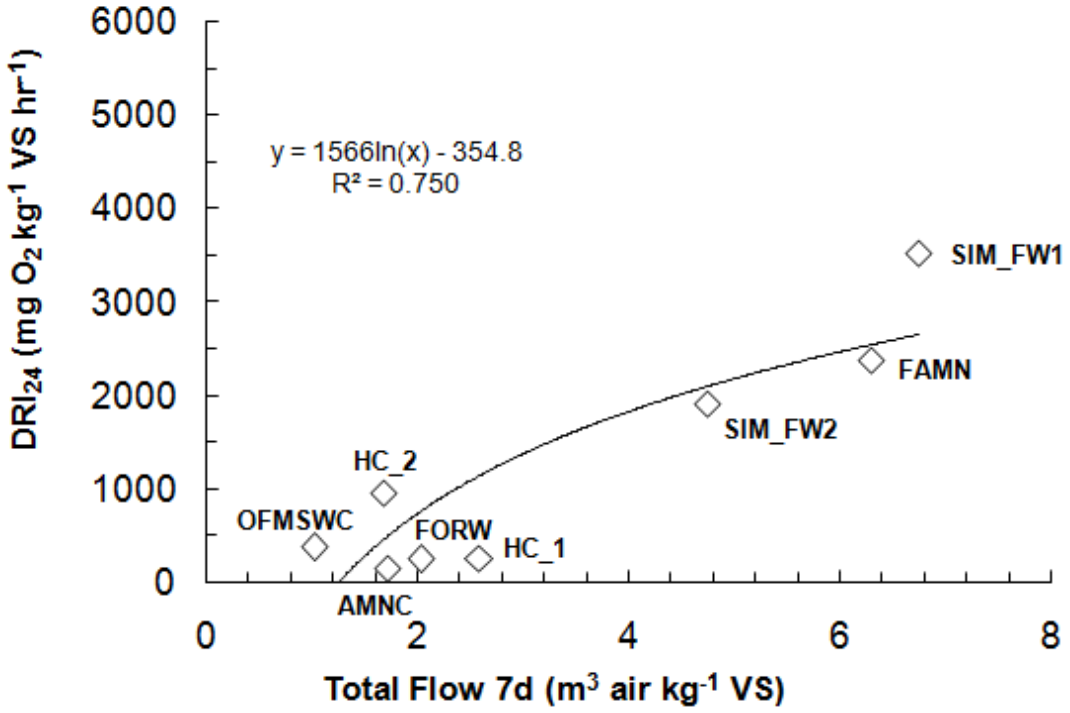

6000
5000
4000
3000
2000
1000
0
DRI24 (mg O2 kg-1 VS hr-1)
y = 1566ln(x) - 354.8
R² = 0.750
SIM_FW1
FAMN
SIM_FW2
HC_2
OFMSWC
FORW
HC_1
AMNC
0
2
4
6
8
Total Flow 7d (m3 air kg-1 VS)

Figure 4

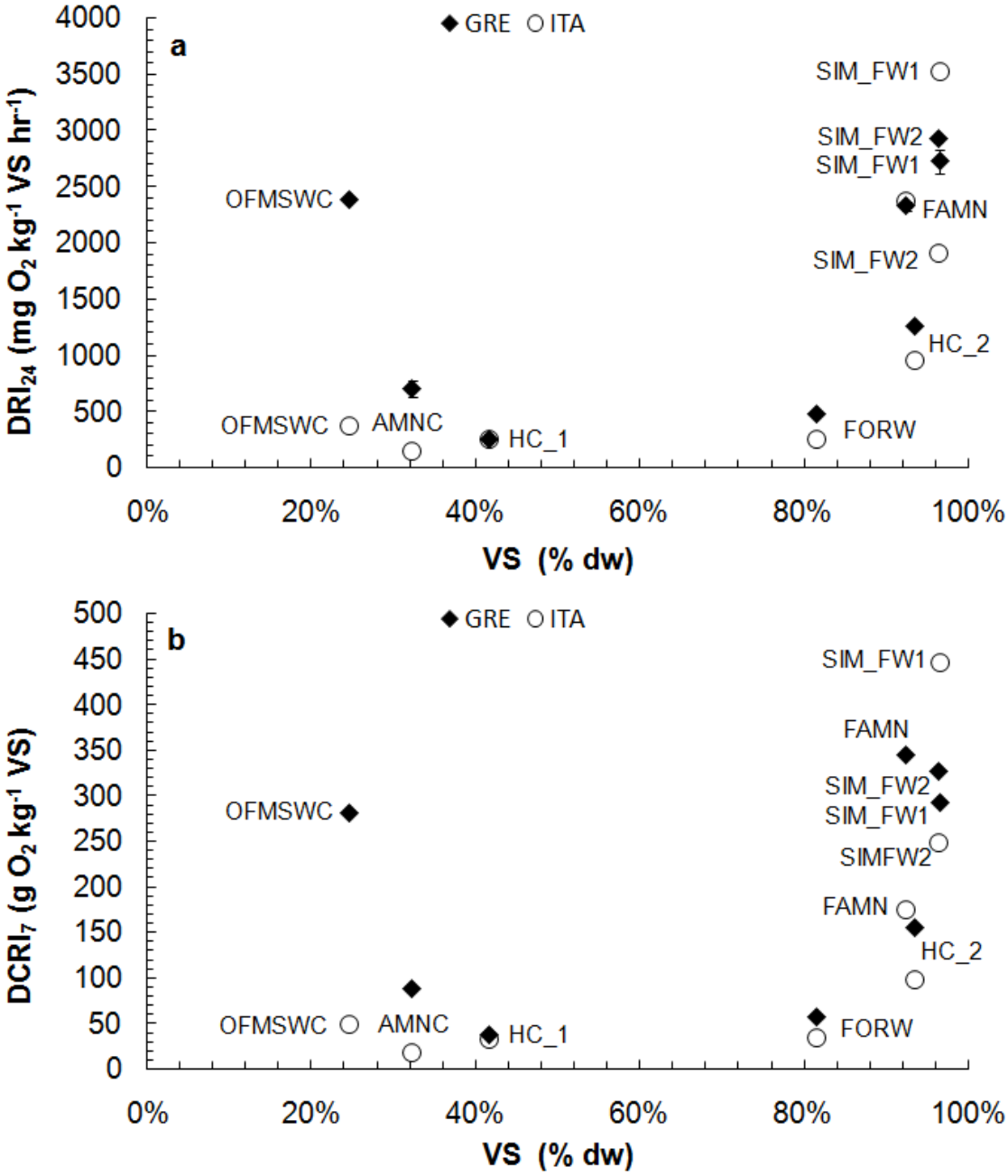
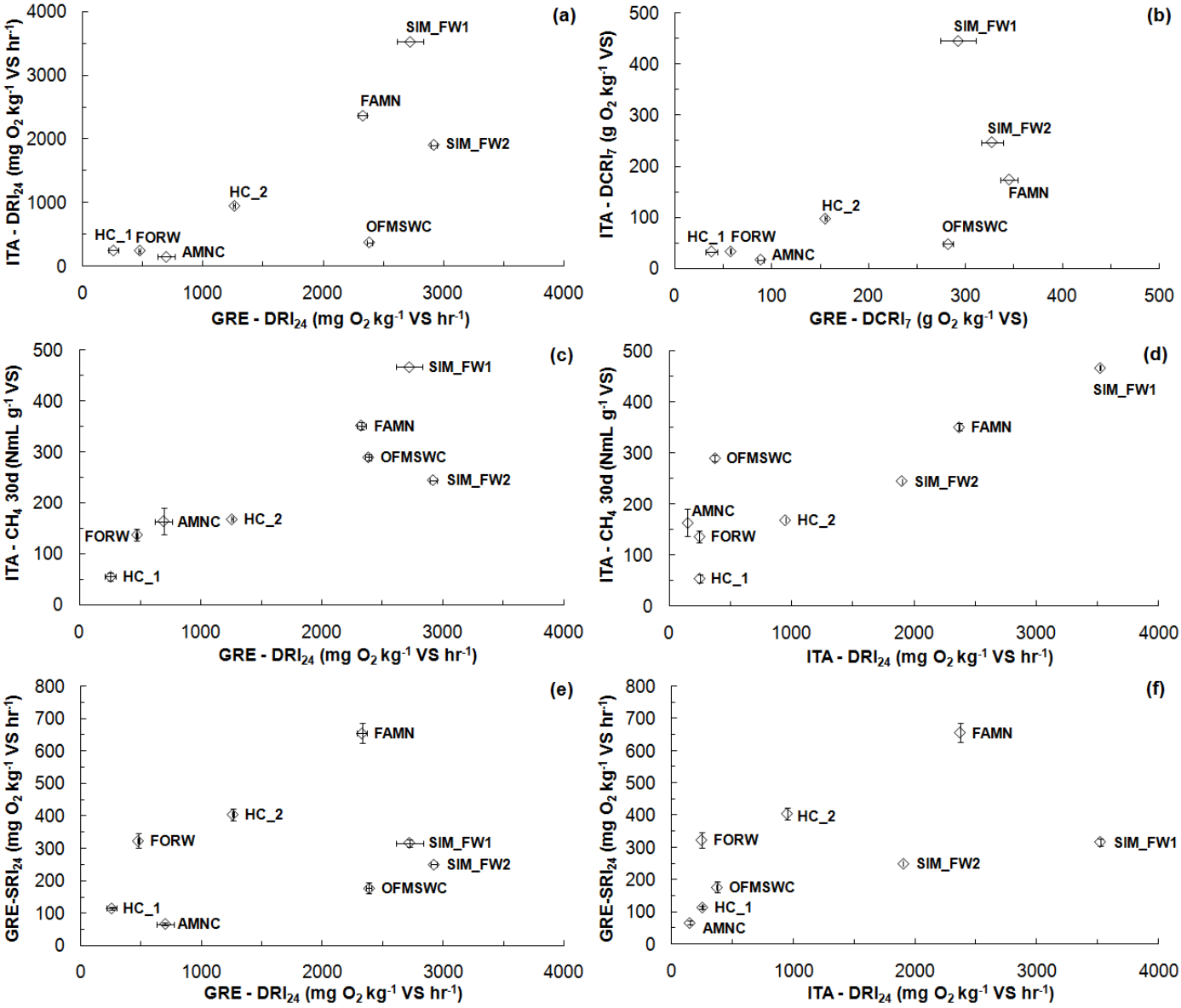

a
GRE
ITA
SIM_FW1
SIM_FW2
SIM_FW1
FAMN
SIM_FW2
HC_2
OFMSWC
OFMSWC
AMNC
HC_1
FORW
DRI24 (mg O2 kg-1 VS hr-1)
VS (% dw)
b
GRE
ITA
SIM_FW1
FAMN
SIM_FW2
SIM_FW1
SIMFW2
FAMN
HC_2
OFMSWC
OFMSWC
AMNC
HC_1
FORW
DCRI7 (g O2 kg-1 VS)
VS (% dw)

Figure 5


(a)
ITA - DRI24 (mg O2 kg-1 VS hr-1)
GRE - DRI24 (mg O2 kg-1 VS hr-1)
SIM_FW1
FAMN
SIM_FW2
HC_2
OFMSWC
HC_1
FORW
AMNC
(b)
ITA - DCRI7 (g O2 kg-1 VS)
GRE - DCRI7 (g O2 kg-1 VS)
SIM_FW1
SIM_FW2
FAMN
HC_2
OFMSWC
HC_1
FORW
AMNC
(c)
ITA - CH4 30d (NmL g-1 VS)
GRE - DRI24 (mg O2 kg-1 VS hr-1)
SIM_FW1
FAMN
OFMSWC
SIM_FW2
AMNC
HC_2
FORW
HC_1
(d)
ITA - CH4 30d (NmL g-1 VS)
ITA - DRI24 (mg O2 kg-1 VS hr-1)
SIM_FW1
FAMN
OFMSWC
SIM_FW2
AMNC
HC_2
FORW
HC_1
(e)
GRE-SRI24 (mg O2 kg-1 VS hr-1)
GRE - DRI24 (mg O2 kg-1 VS hr-1)
FAMN
HC_2
FORW
SIM_FW1
SIM_FW2
OFMSWC
HC_1
AMNC
(f)
GRE-SRI24 (mg O2 kg-1 VS hr-1)
ITA - DRI24 (mg O2 kg-1 VS hr-1)
FAMN
HC_2
FORW
SIM_FW1
SIM_FW2
OFMSWC
HC_1
AMNC