Soil Biology and Biochemistry 120 (2018) 222–229

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Impact of single and binary mixtures of phenanthrene and N-PAHs on microbial utilization of ^{14}C-glucose in soil

Ihuoma N. Anyanwu[a,b], Kirk T. Semple[a,*]

[a] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom
[b] Department of Biological Sciences, Federal University Ndufu-Alike Ikwo, P.M.B 1010, Abakaliki, Ebonyi State, Nigeria

ARTICLE INFO

Keywords:
N-PAHs
Phenanthrene
^{14}C-biomass uptake
Mineralisation
k_{EC} coefficient

ABSTRACT

Microbes are susceptible to contaminant effects, and high concentration of chemicals in soil can impact on microbial growth, density, viability and development. The impact of single and binary mixtures of phenanthrene and its nitrogen-containing polycyclic aromatic hydrocarbon analogues (N-PAHs) on microbial metabolism of ^{14}C-glucose in soil was measured over a 90 d soil-contact time. Impacts were assessed by measuring the rates and mean overall extents of mineralisation (%), as well as the incorporation of ^{14}C-glucose into the microbial biomass. The result revealed that the extents of ^{14}C-glucose mineralisation were consistently greater in N-PAH amended soils than the control and phenanthrene soils with increased incubations. This indicates a trend of increasing diversion of C from biosynthesis to maintenance requirement by soil microorganisms. Furthermore, biomass uptake in the amended soils showed reduced substrate utilization (fixed-k_{EC}), suggesting that N-PAHs decreased the amount of substrate-C that was incorporated into the microbial biomass. This however, signifies that N-PAHs imposes oxidative stress on soil microbial community.

1. Introduction

The importance of microbial activity in the cycling of organic matter and regulating active nutrient pools suggests that the effects of stress on microbial community will fundamentally impact on crops, natural vegetation and ecosystem productivity (Killham, 1985; Anyanwu and Semple, 2016a; Siles and Margesin, 2017). Soil microorganisms are very sensitive to environmental stress or change, and this often results in the diversion of carbon from biosynthesis to maintenance of cells (Bargett and Saggar, 1994; Anyanwu and Semple, 2016a). Thus, soil microbial biomass measurements are important in ascertaining the extent of chemical stress and/or disturbance on soil ecosystem and the time dependence of microbial recovery. Most studies have used respiration rate (Fournier et al., 1992; Nakamoto and Wakahara, 2004; Anyanwu and Semple, 2016a; Sun et al., 2017; Xu et al., 2017) and changes in biomass (Anyanwu and Semple, 2016a; Mehnaz et al., 2017; Siles and Margesin, 2017). Using a^{14}C-substrate, the influence of synthetic and organophosphate sheep dip formulations (Boucard et al., 2008), pesticides (Fournier et al., 1992), heavy metals (Bargett and Saggar, 1994; Bogomolov et al., 1996), sewage sludge (Flieβbach et al., 1994; Witter and Dahlin, 1995) and the ratio of ^{14}C-biomass-incorporated with ^{14}C-respired (Sparling and West, 1988; Sparling et al., 1990; Gunina et al., 2017), have been determined on soil microbial activity. The approach of using ^{14}C-glucose as a substrate to determine the ratio of respired-C, to biomass-incorporated C, has shown that microorganisms in contaminated soils are less efficient in the utilization of substrates for biomass synthesis and spend more energy in the maintenance requirements (Bargett and Saggar, 1994; Witter and Dahlin, 1995; Anyanwu and Semple, 2016a; Gunina et al., 2017). Thus, leading to a decrease in the ratio, increases in stress, faster respiration, reduced efficiency of fresh substrate incorporation into new soil microbial biomass and increased microbial turnover in contaminated soils (Fliebβach et al., 1994; Bargett and Saggar, 1994; Witter and Dahlin, 1995; Boucard et al., 2008; Gunina et al., 2017; Bore et al., 2017). These studies have revealed that the growth, activity and physiological conditions of soil microbial community may be altered and/or destroyed by the presence of contaminants.

Persistent contaminants are of particular concern due to their toxicity and widespread pollution that has occurred during production, spills, combustion and disposition (Van Beelen and Doelman, 1997; Anyanwu and Semple, 2015a); examples include metals, pesticides and polycyclic aromatic hydrocarbons (PAHs). However, for sustainable environmental policies and regulations, risk assessment of other persistent contaminants such as, the nitrogen-containing polycyclic aromatic hydrocarbons (N-PAHs) in the environment is of great importance. N-PAHs are chemicals present in most contaminated sites

* Corresponding author.
E-mail address: k.semple@lancs.ac.uk (K.T. Semple).

https://doi.org/10.1016/j.soilbio.2018.02.009
Received 21 September 2017; Received in revised form 8 February 2018; Accepted 12 February 2018

worldwide and represent two-thirds of known organic xenobiotic chemically synthesized (Rajasekhar et al., 2000; Anyanwu and Semple, 2015a). For example, they are used as industrial solvents, dyes, explosives, pharmaceuticals and pesticides (Kaiser et al., 1996). The US Environmental Protection Agency (USEPA) and International Agency for Research on Cancer (IARC) classified N-PAHs as probable human carcinogens (IARC, 2012). Furthermore, many of these N-PAHs are antimicrobial (Vance et al., 1987; Ferraz et al., 2017); therefore, their accumulation is a major threat to microbes because they have the potency of inducing oxidative stress to soil microorganisms and other biotas.

Despite the widespread uses of N-PAHs, and previous N-PAHs studies in literature (Anyanwu and Semple, 2015a, 2015b; 2016a; Anyanwu et al., 2017), there has not been information of their impacts on microbial utilization of ^{14}C-glucose and/or synthesis of cell biomass in soil. Functionally, microbes can act as relevant indicators of environmental pollution; as a result, there is great need to assess the impact of N-PAHs on soil microbial metabolism and biosynthesis of cell biomass. In this study therefore, the impact of single and binary mixtures of phenanthrene and its nitrogen-containing analogues on microbial utilization of ^{14}C-glucose was investigated over a 90 d incubation period in soil using respirometric assays.

2. Materials and methods

2.1. Chemicals

Phenanthrene (Phen), 1,10-phenanthroline (1,10-Phen), 1,7-phenanthroline (1,7-Phen), 4,7-phenanthroline (4,7-Phen) and benzo[h] quinoline (B[h]Q) and radiolabelled ^{14}C-glucose were obtained from Sigma-Aldrich, UK. Goldstar liquid scintillation cocktails were supplied by Meridian Biotechnologies Ltd, UK.

2.2. Soil preparation

A pristine agricultural soil from Myerscough, UK, collected from the top layer of field under pasture, from a depth of approximately 5–20 cm was prepared for the study (n = 3). The soil texture was sandy-loam (19.5% clay, 60.4% sand, 20.0% silt), with organic matter content of 2.7%; total nitrogen of 0.14%; total organic carbon of 1.6% and pH 6.5. The soil was thoroughly homogenized, air dried at room temperature and sieved with 2 mm mesh size. The soil was rehydrated with deionised water back to 45% water holding capacity (WHC) and amended with phenanthrene and the N-PAH analogues as described in Doick et al. (2003). Soil samples were placed in bowls: $^1/_3$ (100 g; n = 3) were amended with phenanthrene and four N-PAH standards (benzo[h]quinoline, 1,10-phenanthroline, 1,7-phenanthroline or 4,7-phenanthroline) dissolved in acetone to give concentration of 100 mg/kg. The amended soils were kept in the fume hood for 3 h to allow the carrier solvent volatilize, after which the soils were mixed with the remaining ⅔ (200 g). Blanks were prepared using un-amended soils. Soils amended with acetone only were also prepared to serve as a control. The amended soils were kept in amber glass jars and aged in the dark at 21 ± 1 °C for 1, 30, 60 and 90 d. Soil moisture content was checked regularly and lost water was replenished with deionised water. After each ageing time (30 d interval), soils were analysed for microbial-substrate-mineralisation and biomass uptake. Extractability of phenanthrene and the N-PAH analogues from soil over time, and their percentage recoveries has been reported by Anyanwu and Semple (2015b, 2016a) (Table 1).

2.3. Mineralisation o f^{14}C-glucose in soil

The ability of indigenous soil microorganisms to mineralise ^{14}C-glucose to $^{14}CO_2$ was assessed at 1, 30, 60 and 90 d contact time. Respirometric assays were carried out in modified 250 ml Schott bottles incorporating a Teflon-lined screw cap containing 1 M NaOH to trap any $^{14}CO_2$ (Reid et al., 2001). A slurry system with a solid: liquid ratio of 2:1 (20 g soil: 10 ml sterile water) was used to ensure complete $^{12/14}$C-glucose distribution. Standards were prepared in sterilized deionised water and delivered to give a^{12}C-glucose concentration of 3 mM glucose solution with an associated ^{14}C-activity of 800 Bq per respirometer. Controls were also prepared. Respirometers were shaken at 100 rpm on an orbital shaker (Janke and Kunkel, IKA®-Labortechnik KS 510D), in the dark at 21 ± 1 °C. Sampling was carried out every 1, 2, 4, 6, 8, 12, 24 h and 2, 3, 4, 5 d with the vials containing trapped $^{14}CO_2$. Goldstar liquid scintillation cocktail was added to the vials. The vials were stored in the dark for 24 h before sample quantification was carried out by liquid scintillation counting (LSC) using standard calibration and quench correction techniques (Reid et al., 2001).

2.4. Uptake of ^{14}C glucose into microbial biomass

After each 5 d incubation, soil samples from respirometers were divided into three portions and analysed as follows:

a) *Sample oxidation:* The first sample was oven dried at 30 °C and combusted in a sample oxidizer (Packard 307) to determine the level of ^{14}C-activity remaining (i.e. residual ^{14}C-activity in soil). Soil (1 g), plus 200 µl of combustAid was combusted for 3 min. Carbon-sorb-E (10 ml) and Permaflour-E (10 ml) was used as CO_2 trap and scintillation fluid, respectively. Sample quantification was carried out using LSC.
b) *Un-fumigated extraction:* The second sample (~4 g) was immediately extracted with 0.5 M K_2SO_4 (50 ml, pH 7) by shaking on an orbital shaker at 100 rpm for 30 min. The soil solutions were filtered using Whatman No 1 filter papers and an aliquot of 5 ml supernatant was added to 15 ml scintillation cocktail. The quantification of ^{14}C-activity was carried out using the LSC.
c) *Fumigated extraction:* The third sample (~4 g) was placed in a desiccator and fumigated with ethanol-free chloroform for 24 h to measure the ^{14}C-activity within microbial biomass. After fumigation, the samples were vented to remove chloroform residuals in the soil. After venting, samples were extracted with 0.5 M K_2SO_4, filtered (using Whatman No 1 filter papers) and analysed as per the un-fumigated extract.

2.5. Statistical analysis

The proportion of ^{14}C-glucose incorporated into the microbial biomass was calculated as in Sparling et al. (1990) and Boucard et al. (2008).

^{14}C-flush = ^{14}C-activity in fumigated soil – ^{14}C-activity in un-fumigated soil.

^{14}C-microbial biomass = ^{14}C-flush ÷ $k_{EC.}$

1. A fixed k_{EC} coefficient (0.35) was used to convert C-flush into microbial biomass Sparling et al., 1990; Boucard et al., 2008).
2. Variable k_{EC} coefficients were also calculated from each amendment, at all the ageing times, and the ^{14}C-microbial biomass was recalculated with the new coefficient. This process is based on the assumption that; the calculated ^{14}C-labelled microbial-C is a representative of the total microbial biomass and that all the ^{14}C-activity not taken into account by mineralisation and un-fumigated soil extraction has been incorporated into the microbial biomass with negligible amount of extracellular metabolite (Sparling et al., 1990; Boucard et al., 2008).

kEC = (14C-flush) ÷ (14Cinit.– 14C-respired – 14C-activity in un-fumigated soil).

^{14}C-flush and ^{14}C-microbial biomass were later on expressed as