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Biological N_2 fixation by heterotrophic and phototrophic bacteria in association with straw

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Abstract

Much of the crop residues, including cereal straw, that are produced worldwide are lost by burning. Plant residues, and in particular straw, contain large amounts of carbon (cellulose and hemicellulose) which can serve as substrates for the production of microbial biomass and for biological N_2 fixation by a range of free-living, diazotrophic bacteria. Microorganisms with the dual ability to utilise cellulose and fix N_2 are rare, but some strains that utilize hemicellulose and fix N_2 have been found. Generally, cellulolysis and diazotrophy are carried out by a mixed microbial community in which N_2-fixing bacteria utilise cellobiose and glucose produced from straw by cellulolytic microorganisms. N_2-fixing bacteria include heterotrophic and phototrophic organisms and the latter are

apparently more prominent in flooded soils such as rice paddies than in dryland soils. The relative contributions of N_2 fixed by heterotrophic diazotrophic bacteria compared with cyanobacteria and other phototrophic bacteria depend on the availability of substrates from straw decomposition and on environmental pressures. Measurements of asymbiotic N_2 fixation are limited and variable but, in rice paddy systems, rates of 25 kg N ha^{-1} over 30 days have been found, whereas in dryland systems with wheat straw, in situ measurements have indicated up to 12 kg N ha^{-1} over 22 days. Straw-associated N_2 fixation is directly affected by environmental factors such as temperature, moisture, oxygen concentration, soil pH and clay content as well as farm management practices. Modification of managements and use of inoculants offer ways of improving asymbiotic N_2 fixation.

In laboratory culture systems, inoculation of straws with cellulolytic and diazotrophic microorganisms has resulted in significant increases in N_2 fixation in comparison to uninoculated controls and gains of N of up to 72 mg N fixed g^{-1} straw consumed have been obtained, indicating the potential of inoculation to improve N gains in composts that can then be used as biofertilisers. Soils, on the other hand, contain established, indigenous microbial populations which tend to exclude inoculant microorganisms by competition. As a consequence, improvements in straw-associated N_2 fixation in soils have been achieved mostly by specific straw-management practices which encourage microbial activity by straw-decomposing and N_2-fixing microorganisms.

Further research is needed to quantify more accurately the contribution of asymbiotic N_2 fixation to cropping systems. New strains of inoculants, including those capable of both cellulolytic and N_2-fixing activity, are needed to improve the N content of biofertilisers produced from composts. Developments of management practices in farming systems may result in further improvements in N_2 fixation in the field.

Introduction

Maintenance of adequate levels of soil organic matter is essential for a sustainable, high production of crops. Several countries traditionally have utilised crop residues such as straw to maintain or improve the organic matter content of soil (Ayanaba and Okigbo, 1975; Egawa, 1975). However, intensive agriculture systems, with high inorganic fertiliser inputs, limit the return of crop residues to the soil. Worldwide, most crop residues (including cereal straw, rice straw and sugar cane trash) are lost by burning (Flinn and Marciano, 1984; Ponnamperuma, 1984). Straw contains useful macronutrients such as nitrogen (N), phosphorus and potassium, but in particular, it consists of a large amount of carbon which serves as a substrate for the production of microbial biomass and for biological N_2 fixation. It appears that there are two ways in which crop residues are used for energy by asymbiotic N_2-fixing bacteria, i.e. directly through the use of some hemicellulose components (Halsall et al.,1985; Ladha et al.,1986a) or indirectly through the use of products of straw decomposition (Jensen and Swaby, 1941; Lynch and Harper, 1983; Roper and Halsall, 1986). This paper reviews published work on N_2 fixation associated with crop residues under controlled laboratory conditions and in the field. Strategies to increase the efficiency of use of crop residues for N_2 fixation are presented.

Asymbiotic diazotrophs

Several groups of asymbiotic N_2-fixing bacteria have been identified in soils and flooded systems and those genera which include N_2-fixing species are listed in Table 1. The heterotrophic diazotrophs depend on carbon, e.g. from straw, for energy and the most common isolates from soils are (*Azotobacter*, *Azomonas*, *Beijerinckia* and *Derxia*, *Clostridium* and *Bacillus*, *Klebsiella* and *Enterobacter*, and *Azospirillum*, *Desulfovibrio* and *Desulfotomaculum*) (Havelka et al., 1982; Roper and Halsall, 1986). Autotrophic bacteria generally derive their energy from photosynthesis (Havelka et al., 1982).

Straw components and their direct utilisation by diazotrophs

Cellulose and hemicellulose are major structural polysaccharides in straw and comprise between 50–70% of its dry weight (Harper and Lynch, 1981; Theander and Åman, 1978). The low molecular weight carbohydrates range from 0.3 to 1.3% of the dry weight and are mostly fructose, glucose, sucrose and sugar alcohols (arabinitol and mannitol) (Theander and Åman, 1984). A wide range of low molecular weight carbon compounds can be utilised as carbon and energy substrates by diazotrophs. However, very few organ-

Table 1. Genera which include asymbiotic N_2-fixing organisms from soils and flooded systems (from Dalton, 1980; Havelka et al., 1982; Staley, 1989; Stewart, 1980)

Heterotrophic bacteria	Autotrophic bacteria
Aerobic	Cyanobacteria
Azotobacter	Heterocystous filamentous forms
Azomonas	*Anabaena*
Beijerinckia	*Anabaenopsis*
Derxia	*Aulosira*
	Cylindrospermum
Microaerobic	*Nostoc*
Azospirillum	*Calothrix*
Aquaspirillum	*Fischerella*
Thiobacillus	*Hapalosiphon*
Pseudomonas	*Scytonema*
Xanthobacter	*Tolypothrix*
Rhizobium	*Stigonema*
Methylosinus	*Westiella*
Methylococcus	
Mycobacterium	Non-heterocystous filamentous forms
	Lyngbya
Facultative anaerobic	*Phormidium*
Klebsiella	*Plectonema*
Erwinia	*Oscillatoria*
Enterobacter	*Pseudanabaena*
Citrobacter	
Escherichia	Unicellular forms
Bacillus	*Gloeothece*
	Cyanothece
Anaerobic	*Synechococcus*
Desulfovibrio	
Desulfotomaculum	Other phototrophic bacteria
Clostridium	*Rhodospirillum*
	Rhodopseudomonas
	Rhodomicrobium
	Chromatium
	Thiocystis
	Chlorobium

isms have the dual ability to utilise cellulose and fix N_2. The only reports of cellulolytic, N_2-fixing bacteria are a marine bacterium isolated from a shipworm (Greene and Freer, 1986; Waterbury et al., 1983) and four strains of anaerobic bacteria isolated from forest soil and fresh-water mud (Leschine et al., 1988).

Xylan, which is the predominant component of hemicellulose, can be utilised by *Azospirillum* (*A. lipoferum* and *A. brasilense*) as the sole carbon source for N_2 fixation (Halsall et al., 1985; Ladha et al., 1986a). Halsall et al. (1985) also measured nitrogenase activity when xylan was replaced by powdered, gamma-irradiated straw, suggesting the use by diazotrophs, of xylan, pectins, and other easily available carbohydrates of low molecular weight in the straw. Ladha et al. (1986a) reported N_2 fixation by a range of N_2-fixing bacteria isolated from the rice rhizosphere (*Azospirillum lipoferum*, *Pseudomonas diazotrophicus*, *Enterobacter cloacae* and *Klebsiella planticola*) in the presence of hydrogen peroxide-treated rice straw,

again suggesting the utilisation of xylan as a sole carbon source. The pretreatment of straw with hydrogen peroxide frees its components and partly solubilises lignin, hemicellulose and the alcohol extractable fraction. Ladha et al. (1986a) found several hundred times higher nitrogenase activity by *Azospirillum lipoferum* in the presence of hydrogen peroxide-treated straw than in untreated straw. These reports suggest that the enzyme xylanase is much more widespread among diazotrophs than has been assumed previously.

Aerobic and anaerobic cellulose degradation

Since most N_2-fixing bacteria are unable to utilise cellulose directly as a substrate for N_2 fixation, cellulose must first be degraded to simpler intermediates before being utilised by diazotrophs. In nature, most cellulose is degraded aerobically and the final product is CO_2. The degradation occurs in two steps: i) degradation by cellulolytic microorganisms (primary microorganisms) to cellobiose and glucose, and ii) utilisation of cellobiose, glucose and other free sugars by secondary microorganisms which are unable to hydrolyse cellulose. In anaerobic systems, such as in flooded soils, methane is produced from decomposing organic matter and, the cellulose is oxidised to proprionate, butyrate, lactate, acetate, molecular hydrogen, and CO_2. Anaerobic decomposition of straw in flooded soil is reviewed in detail by Yoshida (1975), Neue and Scharpenseel (1984) and Watanabe (1984).

Organisms involved in the aerobic decomposition of straw include a broad range of bacteria, actinomycetes, fungi, protozoa, nematodes and worms (Chatterjee and Nandi, 1981; Dickinson, 1974; Imshenetsky, 1967; Veal and Lynch, 1984; Zeikus, 1981), and the populations of these organisms increase in response to the addition of straw (Doran, 1980). Generally, there is a succession of microorganisms involved in decomposition (Swift, 1982) with the primary decomposition being attributed largely to the microflora, which in turn, are consumed by the soil fauna (Forbes, 1974).

Characterisation of cellulolytic microorganisms in flooded soil is limited (Watanabe and Furusaka, 1980). However, there are reports of the occurrence of aerobic fungi and bacteria, and anaerobic bacteria that decompose cellulose (Araragi and Tangcham, 1979; Saito et al., 1977a, b; Vostrov and Dolgikh, 1970). Among the anaerobic cellulolytic bacteria, *Clostridium dissolvens* has been found in flooded soil sown to rice (Saito et al., 1977a, b).

N_2 fixation in soils by bacteria using products of straw decomposition

Virtually all diazotrophic heterotrophs from dryland and flooded soils can utilise the products of cellulose decomposition including carbohydrates and some organic acids and alcohols (Jensen, 1981; La Rue, 1977; Rao, 1978; Roper and Halsall, 1986). Phototrophic nonsulphur purple bacteria which occur in fairly high numbers in flooded soil (Kobayashi et al., 1967; Ladha et al., 1987; Watanabe et al., 1978), also can utilise products of cellulose degradation (Kobayashi, 1982; Stanier et al., 1974).

N_2 fixation in flooded soils

Although the flooded rice ecosystem remains continuously flooded during crop growth, and is referred to as anaerobic, it actually consists of aerobic and anaerobic phases in close proximity to each other. This ecosystem has two major zones – the rhizosphere and the nonrhizosphere. The rhizosphere, the zone in close proximity to the roots, has intense microbial activity which is supported mostly by the release of carbon compounds from the root system. Based on physicochemical properties, the nonrhizosphere zone can be divided into four environments – the floodwater, the oxidised soil layer, the reduced soil layer and the subsoil. The floodwater and the oxidized soil layer are photic environments with a positive redox potential, while the reduced soil layer is nonphotic with a redox potential that is generally negative. The soil below the plough pan layer is either aerobic or anaerobic depending on the drainage.

N_2 fixation has been reported in flooded soil amended with straw (Barrow and Jenkinson, 1962; Brouzes et al., 1969; Magdoff and Bouldin, 1970; Rice and Paul, 1972). The application of straw to the surface or subsurface layers of flooded soil results in intense microbial activity which leads to high oxygen demand and consumption. As a result, an anaerobic to microaerobic environment, conducive to N_2 fixation by heterotrophic and phototrophic bacteria, is created. It has been suggested that either i) the low molecular weight products of aerobic degradation of polysaccharides in the aerobic zone diffuse into the anaerobic zone where they support N_2 fixation by anaerobic bacteria such as *Clostridium*, or ii) the products of anaerobic degra-

Table 2. Biological N_2 fixation or N gain by straw-amended flooded soil (modified from Roger and Watanabe, 1986)

Amount of soil used (g)	Straw			Incubation time (days)	Quantification method[b]	Efficiency: mg N fixed per g of straw		Reference
	Type	Nature	Amount (g)			Added	Consumed	
5	Barley	Powdered	0.5 (10)[a]	28	Kj	2.2–2.5		Barrow and Jenkinson (1962)
			1.0 (20)	28	Kj	0.8–1.0		
			2.0 (40)	28	Kj	1–2		
			0.72 (15)	14	Kj	1		
				28	Kj	2		
				56	Kj	5		
0.6	Wheat	Powdered	0.006 (1)	28	^{15}N	6.7		Rice et al. (1967)
			0.03 (5)	28	^{15}N and Kj	4.4		
			0.12 (20)	28	Kj	2.3		
0.1 (+ 2 g sand-clay)	Wheat	Powdered	0.12 (20)	28	Kj	2.2	16.1	Rice and Paul (1972)
5	Rice	Powdered	0.05 (1)	30	^{15}N	1.7		Rao (1976)
			0.1 (2)	30	^{15}N	7.07		

[a]Figures in parentheses are straw concentration in percent.
[b]Kj – Kjeldahl, ^{15}N – ^{15}N incorporation.

dation of cellulose in the anaerobic zone diffuse into the overlying aerobic zone to support N_2 fixation by aerobic bacteria (Knowles, 1976).

N_2 fixation, supported by straw in flooded soil, has been evaluated in two types of experiments, i) under laboratory conditions with rather high amounts of straw (equivalent to 1 to 100% of soil, dry weight basis), and ii) under greenhouse or field conditions with lower amounts of straw (equivalent to < 1% of soil, dry weight basis). The first type of experiment probably simulated a composting environment.

Laboratory studies have been reviewed in a paper by Roger and Watanabe (1986). A wide range of values for N_2 fixation and N gain have been obtained by researchers due to differences in the form and amount of straw, the time of incubation, and the methods used for quantification (Table 2). Since the amount of straw used in most of these experiments was very high and practically impossible to apply in the field, data on the amount of N_2 fixation should not be extrapolated on a hectare basis. The most practical way of expressing N_2 fixed could be based on the amount of organic matter added and consumed (Roger and Watanabe, 1986); however, the amount of straw consumed was frequently unrecorded. Based on some published data, N_2 fixed varied from 1 to 7 mg N per g of straw added in 14 to 56 days of incubation (Table 2).

Only a few quantitative data on the amounts of N_2 fixed or N gained following straw application in greenhouse or field conditions are available. Rao (1980) quantified N_2 fixation in flooded soil amended with straw and planted to rice. Chopped straw (5 and 10 t ha^{-1}) was incorporated one month before rice transplanting. Straw amended soil (5 g) was sampled from 0–10 cm depth at tillering and at harvest in the first rice crop and again at tillering in the succeeding rice crop; the soil samples were incubated with $^{15}N_2$ for 30 days. N_2 fixation in the straw-amended soil was 2 to 4 times that of the control without straw. Fixation was highest at the tillering stage of the first crop after which there was a gradual decline, with negligible amounts measured in the control and in the treatment with 5 t ha^{-1} straw in the second crop. The treatment with 10 t ha^{-1} straw still showed appreciable N_2 fixation at the tillering stage of the second rice crop, suggesting a residual effect. Extrapolation of the values of ^{15}N incorporation in straw-amended soil for the 30-day duration, indicated rates of N_2 fixation of about 7 kg ha^{-1} in the unamended soil and an average of 25 kg ha^{-1} in soil with straw amendment, based on a per hectare furrow slice of 0.7×10^6 kg dry soil ha^{-1}.

The figures may have been higher for the whole crop duration. However, these rates of N_2 fixation should be used with caution because the ^{15}N measurements were made under laboratory conditions.

Santiago-Ventura et al. (1986) conducted a N balance pot experiment for three consecutive rice crops and reported about twice the N gain following straw incorporation (equivalent to 10 t ha^{-1}) compared with the control. When the N gain was expressed per g of straw added, it ranged from 2 to 4 mg N fixed g^{-1} straw added. Apart from these quantitative data on N gain and N_2 fixation, several reports of semiquantitative measurements are available (Kimura et al., 1979; Kobayashi, 1982; Ladha et al., 1986b, 1987; Matsuguchi, 1979; Rao, 1976, 1978; Reddy and Patrick, 1979; Rice, 1979; Wada et al., 1979; Yoneyama et al., 1977; Yoo et al., 1982). Time-course measurements of N_2 fixation using acetylene reduction methods (ARA) generally showed stimulation from a few days to about 40 days after straw application, followed by a gradual decline (Ladha et al., 1986b, 1987). Activity was higher during the dry season than during the wet season. The trend of ARA in field studies was more or less similar to that in the laboratory studies (Rao, 1976; Rice and Paul, 1972; Rice, 1979; Yoneyama et al., 1977).

Most laboratory experiments were incubated in the dark permitting only heterotrophic N_2 fixation. On the other hand, greenhouse and field experiments included N_2 fixation by both heterotrophic and phototrophic microorganisms. The relative contributions of N_2 fixation by heterotrophic and phototrophic microorganisms in straw-amended soil are not known. Furthermore, among phototrophic microorganisms, the relative contributions of cyanobacteria (blue-green algae) and phototrophic (photosynthetic or photoorganotrophic) bacteria are not clear. Diverse types of cyanobacteria (Roger and Kulasooriya, 1980) and phototrophic purple nonsulphur bacteria of the family *Rhodospirillaceae* (Kobayashi et al., 1967) are ubiquitous in rice fields. Their growth is stimulated by straw application, although to a lesser extent with cyanobacteria (Matsuguchi, 1979; Yoo et al., 1982), and to a greater extent with the phototrophic bacteria (Kobayashi and Haque, 1971; Ladha et al., 1986a, 1987; Matsugushi and Yoo, 1981; Yoo et al., 1982).

In order to determine the relative contributions of cyanobacteria and phototrophic bacteria to N_2 fixation in flooded soil, Habte and Alexander (1980) used a herbicide (propanil 3', 4'-dichloropropionanilide) to inhibit nitrogenase activity by cyanobacteria. The herbicide had no effect on other N_2-fixing bacteria including phototrophic bacteria. They found significantly higher N_2-fixing activity in the herbicide-treated soil than in the untreated soil after 30 days of rice growth but no difference after 55 days. Their study concluded that the contribution of N_2 fixation by phototrophic bacteria may be as high as that by cyanobacteria; furthermore, it may be higher if cyanobacterial growth is poor. Reddy and Patrick (1979) recorded 3- to 4-fold higher nitrogenase activity in straw-amended soil incubated in the light compared with the dark, and suggested that increased activity was due to phototrophic bacteria. Similarly, Yoo et al. (1982) and Ladha et al. (1987) suspected that phototrophic bacteria are more important than the cyanobacteria in flooded soils. Yoo et al. (1982) suggested that cyanobacterial growth may be inhibited by the increased population of molluscs resulting from straw application. Ladha et al. (1987) recorded a significant increase in the population of phototrophic purple nonsulfur bacteria in soil amended with straw (5t ha^{-1}) in the light, and this increase was greater where the straw was applied at the surface of the flooded soil than where the straw was incorporated into the soil.

In a small-scale laboratory experiment with straw, Kobayashi and Haque (1971) demonstrated a succession of heterotrophic bacteria, phototrophic bacteria and green algae. They found peaks in population sizes of heterotrophic bacteria at about 30 days, followed by phototrophic bacteria at about 60 days, and algae at about 120 days. An explanation for this may be that the growth of the heterotrophic bacteria slowed down due to a decrease in the substrate level and an accumulation of decomposition products which favoured the growth of phototrophic bacteria; in another 3 to 4 weeks, when the C:N ratio of straw declined substantially, the growth of phototrophic bacteria also declined and the growth of green algae took over. Other studies by Matsuguchi and Yoo (1981) found that the surface application of straw to flooded soil promoted growth of the phototrophic bacterium *Rhodopseudomonas* in the first 3 weeks; thereafter, there was a cyanobacterial bloom associated with a very high nitrogenase activity. Despite the prominence of phototrophic microorganisms, Santiago-Ventura et al. (1986) found no significant difference in the N balance of straw-amended soil exposed to light or dark.

Generally, phototrophic purple nonsulfur bacteria occurring in flooded rice soil are not strict anaerobes (Kobayashi, 1982). They can also grow aerobically in the dark, obtaining ATP from a respiratory metabolism

of available organic compounds. On the other hand, under anaerobic conditions in the light, the reactions of photosynthesis provide a potentially unlimited supply of ATP and reductants (Stanier et al., 1974).

Phototrophic purple nonsulfur bacteria in pure culture attain maximum nitrogenase activity under light and anaerobic conditions (Kobayashi, 1982). This environment can be provided in the field by intense, highly oxygen-demanding multimicrobial activities induced by surface application of straw (Kobayashi, 1982; Ladha et al., 1987).

Few quantitative data on N_2 fixation by phototrophic bacteria exist, but the semiquantitative data suggest they have significant potential to contribute N to rice-based agriculture. N_2 fixation by phototrophic bacteria could play an important role in rice fields in situations where the conditions are not favourable for good cyanobacterial growth; this is often the case because of limiting factors such as phosphorus deficiency and grazing by invertebrates (Roger and Kulasooriya, 1980). Habte and Alexander (1980) discussed some additional advantages of phototrophic bacteria compared with cyanobacteria: a) faster growth, b) no competition in light utilisation with rice because they utilise light energy in the far red and infrared regions, c) insensitivity to certain herbicides, and d) detoxification of H_2S.

N_2 fixation in dryland soils

Dryland cropping systems include aerobic and anaerobic sites in the same way as flooded systems, although there are differences in scale and arrangement. Intense microbial activity, during the decomposition of crop residues, results in the development of anaerobic and microaerobic microsites in soils, including surface soil (Greenwood and Goodman, 1964; Hill et al., 1990; Parr and Papendick, 1978), and these sites can support N_2 fixation by a wide range of free-living, diazotrophic bacteria, including anaerobic bacteria.

Nitrogen fixation by asymbiotic bacteria has been observed in artificial systems in soils amended with cellulose (Jensen and Swaby, 1941; Kalininskaya, 1972), wheat straw (Delwiche and Wijler, 1956; Halsall and Gibson, 1986, 1989; Lynch and Harper, 1983; Roper and Smith, 1991), oat straw (Brouzes et al., 1969), maize stalks (Abd-el-Malek et al., 1976) and sugar cane trash (Patriquin, 1982) to mention a few. Measurements of N_2 fixed in artificial systems vary greatly. With cellulose as a carbon source, fixation ranged from 2.5–4.5 mg N g^{-1} cellulose (Kalininskaya, 1972) to 14 mg N g^{-1} cellulose (Jensen and Swaby, 1941). Brouzes et al. (1969) found extremely variable rates of N_2 fixation with 2% oat straw (0–70 μg N g^{-1} soil) which, when extrapolated, gave an estimate of annual fixation of 0–200 kg N ha^{-1} $year^{-1}$. Similarly, huge variations in rates of N_2 fixed (2–155 g N ha^{-1} day^{-1}) were found with sugar cane trash by Patriquin (1982), but he estimated that the total N_2 fixed in association with the trash from a cane crop was of the order of 20 kg N ha^{-1}.

Estimates of N_2 fixed in pure culture systems are also extremely variable. For example, Lynch and Harper (1983) measured a N gain of 11.5 mg N g^{-1} wheat straw consumed after incubation of straw inoculated with a N_2-fixing *Clostridium butyricum* and a cellulolytic *Penicillium corylophilum* for a period of 8 weeks. On the other hand, over 38 days, Halsall and Gibson (1986) calculated an efficiency of N_2 fixation by *Azospirillum brasilense* of 72 and 63 mg N g^{-1} wheat straw utilised, in cultures containing respectively, *Cellulomonas gelida* and *Cellulomonas* sp. as the cellulolytic partner. It is likely that culture conditions as well as the cellulolytic and N_2-fixing partners were significant contributing factors to the variable results. In situ measurements of N_2 fixation associated with wheat straw, made at field sites across eastern Australia by Roper (1983) and Roper et al. (1994a), indicated amounts fixed (based on a C_2H_2:N_2 calibration of 3:1) ranging from 1 kg N ha^{-1} over 31 days to 12.3 kg N ha^{-1} over 22 days. The wheat straw content of the soil ranged from 4.3 – 7.2 t ha^{-1}. All measurements were made under conditions where moisture was not limiting (i.e. field capacity), but soil temperatures ranged from as low as 6°C at night to above 36°C during the day. In a longer term field experiment on a vertisol, nitrogenase activity was detected throughout the 10 month period following straw management after harvest, but the level of N_2 fixation was closely linked to temperature and was highest in the summer and early autumn months (Roper et al., 1989). This means that, even if rates of asymbiotic diazotrophic activity are low, particularly in the winter months, there is still potential for a significant input of N over a period of time. In another system, in situ asymbiotic N_2-fixing activity was observed in association with lucerne and its residues, and it was estimated that this amounted to between 3 and 10 kg ha^{-1}, or 3–10% of the total (symbiotic + asymbiotic) N_2 fixed, over a single growing season in south eastern Australia (Roper et al., 1994b). In situ studies with decomposing green sugar cane trash indicated that the highest levels of N_2

fixation were 1.5 kg ha^{-1} $week^{-1}$ (Chapman et al., 1992). All these rates of asymbiotic N_2 fixation are low in comparison to legume systems (Peoples et al., 1995). However, because N contained in the microbial biomass of N_2-fixing bacteria is readily available as a source of N (Lethbridge and Davidson, 1983), it is likely that N_2 fixed asymbiotically can make a significant contribution to a following crop.

Factors affecting N_2 fixation associated with straw

Straw-associated N_2 fixation is modified by mineral N, temperature, moisture, oxygen concentration, soil characteristics and straw management. Both N_2 fixation and straw decomposition are affected directly by these factors, but N_2 fixation is also affected indirectly through straw decomposition. For example, in a field experiment, Roper (1983) observed a positive correlation ($r = + 0.98$; $p < 0.01$) between nitrogenase activity and wheat straw decomposition.

Cereal straw usually has a high C:N ratio (> 70) and therefore, its decomposition may be slow because of the requirement for N by straw-decomposing microorganisms. Straws with higher N contents decompose more quickly than those containing less N (Zielinski, 1980) and when Smith and Peckenpaugh (1986) studied the decomposition of 23 cereal straws they found a direct correlation ($r = + 0.84$, $p < 0.01$) between the N content of the straw and its decomposition. The addition of mineral N to soils amended with straws with high C:N ratios, increases the rate of straw decomposition (Barder and Crawford, 1981; Novak, 1972; Sain and Broadbent, 1977), but it can also decrease the rate of N_2 fixation (Cejudo and Paneque, 1986; Shintani, 1987) through its inhibitory effects on the nitrogenase enzyme (Knowles and Denike, 1974). Both inhibitory and stimulatory effects of mineral N on straw-associated N_2 fixation have been reported (Charyulu and Rao, 1979; Matsuguchi and Yoo, 1981; Rao, 1976) and this indicates that it is necessary to balance the input of mineral N in order to increase straw decomposition without compromising N_2 fixation.

Both straw decomposition and N_2 fixation are significantly affected by temperature. Pal et al. (1975) and Waksman and Gerretsen (1931) showed that straw decomposition increased with increasing temperatures between 7 and 37°C. Roper (1985) observed decomposition at temperatures up to 50°C with the highest activity in the range between 25 and 45°C. Nitrogenase activity, in the field, was found to be positively correlated ($r = + 0.71$, $p < 0.05$) with temperature (Roper, 1983). In glucose-amended soils in the laboratory, Brouzes and Knowles (1973) showed that 37°C was the optimum temperature for nitrogenase activity, whereas Roper (1985) found maximum activities within the range of 20–35°C in soil from one site and 25–45°C in soil from a second site; these temperature ranges reflected the differences in climate between the 2 sites and suggested some adaptation of the microbial populations to the temperatures of their environment.

Moisture and oxygen are interrelated factors and their effects are difficult to separate. Because straw decomposition involves an enormous variety of microorganisms, decomposition can occur over a wide range of moistures, and has been observed in soils as dry as 30% field capacity and up to waterlogged conditions (Roper, 1985). Sorensen (1974) observed that repeated air drying and rewetting of soils resulted in the best rates of decomposition of plant material. With the sensitivity of the nitrogenase enzyme to oxygen and the dependence, at least in part, on moisture to reduce oxygen levels, N_2 fixation is more sensitive to moisture contents. Generally, laboratory incubation studies with soil plus straw have indicated that the highest levels of N_2 fixation occur under waterlogged anaerobic conditions (Brouzes et al., 1969; Rao, 1976; Rice et al., 1967). In laboratory studies with glucose, Roper (1985) found that moistures of between 1.5 and 2 times field capacity resulted in the best rates of N_2 fixation, but the lowest moisture content which supported nitrogenase activity varied according to the characteristics of the soil. Soils containing clays develop microsites of low oxygen tension more readily than sandy soils and N_2 fixation has been observed in clay soils at moistures below 50% field capacity (Roper, 1985). Undisturbed soils in the field support nitrogenase activity at lower moisture contents than in the laboratory, probably because microsites of low oxygen tensions are preserved. In field observations, Roper (1983) found a positive correlation between nitrogenase activity and moisture content, below field capacity, and detected activity at moistures as low as 25% field capacity.

Under uniform conditions of soil moisture and temperature, different soils with similar histories of straw retention produce different rates of nitrogenase activity. pH and clay content are probably the most significant characteristics which modify microbial activity in soils. Roper and Smith (1991) found that in sand

culture systems, with microorganisms extracted from soils sampled from wheat-growing areas, straw decomposed efficiently over a wide range of pH, reflecting the broad range of microflora responsible for decomposition. However, in the same experiments, N_2-fixing bacteria had a much more restricted pH range for activity and preferred a pH close to neutral regardless of the pH of the soils from which the N_2-fixing bacteria were derived, indicating that N_2-fixing populations are not always suited to their soil environment. Soil pH controls the uptake of nutrients (Stotzky, 1972) and can restrict the range of N_2-fixing genera such as *Azotobacter* in soil (Alexander, 1961).

Clays affect both straw decomposition and N_2 fixation in soils. Christensen (1987) found that decomposition of straw was higher in a sandy soil than in a clay soil. However, it appears that the type of clay is important and montmorillonite depresses decomposition (Novakova and Sisa, 1984; Roper and Smith, 1991) whereas kaolinite enhances decomposition (Novakova and Sisa, 1984). The restriction of decomposition by some clays can be attributed to the inhibition of respiration by the fine colloids, but this in turn favours the development of microaerobic and anaerobic microsites for nitrogenase activity. The addition of montmorillonite to sand cultures containing microorganisms extracted from soils resulted in enormous increases in N_2-fixing activity and extended the pH range of activity (Roper and Smith, 1991). At the pure culture level, Macura and Pavel (1959) found that N_2 fixation by *Azotobacter* sp. was increased significantly by the presence of montmorillonite. Clays are highly reactive colloidal particles (Baver, 1956) and interact strongly with microorganisms (Marshall, 1975). Clays concentrate nutrients at their surfaces, where microbial activity takes place, and modify local pH (Stotzky, 1972). This may explain the intensified nitrogenase activity and the extension of N_2 fixation to a broader pH range in the presence of clay compared with systems without clay.

Straw management has a significant effect on straw decomposition. Cogle et al. (1987), Douglas et al. (1980), Roper et al. (1989) and Summerell and Burgess (1989) all found that straw decomposed more rapidly when incorporated into the soil than when left on the surface as a mulch. This means that products of decomposition from incorporated straw are more readily available for use by N_2-fixing bacteria in soils. For example, in simulated laboratory experiments, Patriquin (1982) observed a larger and earlier peak of nitrogenase activity in soils with incorporated sugar cane trash than in soil with mulched trash. In the field, Roper et al. (1989) and Roper et al. (1994a) found that nitrogenase activity was best with straw incorporation and that activity decreased in the order straw incorporated > straw mulched > no-tillage. They also showed that the type of incorporation of stubble was important and that straw which was smashed and mixed lightly with the soil near the surface produced significantly better nitrogenase activity than soil in which the straw was incorporated throughout the plough layer (Roper et al., 1989). It is likely that the surface mixing of the straw with soil provided sufficient soil-straw contact, and hence microorganism-straw contact, as well as good aeration to encourage decomposition. In addition, concentration of decomposition products and intensified microbial activity near the surface provided an opportunity for the development of anaerobic and microaerobic microsites (Greenwood and Goodman, 1964).

Ways to enhance straw-associated N_2 fixation

There may be two different situations where the value of plant residue, such as straw, is enhanced as a substrate for N_2 fixation: a) where straw is applied directly to soil, and b) where straw is first allowed to decompose and is used as a compost. One strategy to obtain higher N_2 fixation is to inoculate with efficient microorganisms. Soils maintain stable populations of large numbers of diverse microorganisms which generally resist the establishment of inoculated microorganisms because the inoculants compete poorly with the indigenous population (Ladha, 1986; Nayak et al., 1986). Straw, on the other hand, may not have such established populations of microorganisms and hence, may offer a better chance for the survival of inoculated microorganisms. Therefore, the introduction of selected microorganisms to heaps of straw such as composts or surface straw may be a more workable strategy.

After crop harvest, farmers may pile the straw into heaps in the field to burn. By inoculating with a combination of diazotrophic and cellulolytic microorganisms, the straw could be converted to an efficiently decomposed biofertiliser (compost) that could be incorporated easily into the soil. Composting is essentially a microbiological process. Its efficiency depends on the presence of suitable numbers and kinds of diazotrophic and cellulolytic microorganisms, the composition of straw (C:N ratio), temperature, moisture and aeration. As discussed earlier, cellulolysis and N_2 fix-

ation generally are not combined in the same microorganism. The cellulolytic or primary microorganisms degrade cellulose to cellobiose and glucose. The N_2-fixing or secondary microorganisms, which are unable to hydrolyse cellulose, use cellobiose, glucose, and other free sugars as energy sources. The secondary microorganisms, though dependent on the primary microorganisms, also aid the cellulolytic microorganisms by supplying growth factors (including combined N) and removing free sugars (which normally inhibit cellulose degradation).

In the past, more attention was directed towards the selection and use of cellulolytic microorganisms rather than both cellulolytic and N_2-fixing microorganisms (Subba Rao, 1982) with the result that, during the course of composting, the cellulose and hemicellulose frequently was reduced without any appreciable gain in N (Inoko, 1984). For example, after sampling 105 composts, Inoko (1984) measured on average only 3.9 kg N per t of compost. Yadav and Subba Rao (1980) reported an average increase in N from 0.7% of wheat straw to just 1.6% N in the compost after 12 weeks and following a weight loss of 50%. This represented a gain in total N of 1 kg N t^{-1} straw used. These values are much lower than those obtained in the laboratory experiments reported in Table 2. The major difference in conditions between small-scale laboratory and medium to large-scale compost experiments is possibly the level of aeration. Composting often proceeds under more aerobic conditions with fungi rapidly and completely degrading polysaccharides to CO_2, allowing negligible accumulation of simple carbohydrates.

More recently, co-culture of suitable cellulolytic and N_2-fixing strains has been used in laboratory experiments to improve the efficiency of straw-associated N_2 fixation. Harper and Lynch (1984) found that, in straw decomposition/N_2 fixation systems, the cellulolytic population was dominated by aerobic fungi while the major N_2-fixing organisms were anaerobic bacteria. Based on this finding, Harper and Lynch (1986) inoculated wheat straw with an aerobic, cellulolytic *Trichoderma harzianum* and an anaerobic, N_2-fixing *Clostridium butyricum* and obtained N gains of up to 2 mg N fixed g^{-1} straw used over 4 weeks.

As reported earlier, even higher levels of N_2 fixation were measured by Halsall and Gibson (1985, 1986). Using a sterile sand-straw system with *Cellulomonas gelida* strain 2480 and *Azospirillum brasilense* strain Sp7 (or *Bacillus macerans* strain III), Halsall and Gibson (1985) obtained an efficiency of up to 19 mg N fixed g^{-1} straw consumed over a 30-day period. Subsequently, using the same system with *C. gelida* 2480 (or *Cellulomonas* sp. CSl-17, a mutant strain) and *A. brasilense* Sp7, Halsall and Gibson (1986) reported an efficiency of up to 72 mg N fixed g^{-1} straw consumed after 38 days incubation. *Azospirillum* is a microaerobic diazotroph that can produce xylanase allowing it to use hemicellulose (Halsall et al., 1985). *Cellulomonas* CSl-17 is a mutant strain selected after UV mutagenesis for improved cellulase production and for reduced sensitivity to repression inhibition by cellobiose or glucose (Choudhary et al., 1980). Both *Cellulomonas* strains were able to degrade cellulose at wide ranges of oxygen concentration, but the mutant strain CSl-17 accumulated higher amounts of reducing sugars which were then available for use by the diazotrophic bacteria (Halsall and Gibson, 1986).

The results clearly suggest a potential for improving straw-associated N_2 fixation by the use of selected microbial strains. Lynch (1983) suggested that the efficiency of straw associated N_2 fixation in composts could be increased to as much as 15 mg N g^{-1} total straw by inoculating with suitable microbial strains and, when added to the N already present in the straw, this would represent a total N value of 20 kg N t^{-1} straw. The results of Halsall and Gibson (1986) suggest the target for improvement could be even higher. However, because the ability of inoculants to compete with indigenous microbial populations is limited (Ladha, 1986; Nayak et al., 1986), inoculation is only likely to be successful in composts without established microbial populations.

Other approaches to improve the efficiency of N_2 fixation associated with straw are management of straw and manipulation of soil properties. As indicated earlier, pH near neutral favours N_2 fixation and improvements in nitrogenase activity have been achieved by liming of the soil. For example, in situ measurements in forest systems by Jones and Bangs (1985) showed that raising the pH of the soil to 6 from between 4 and 5 more than doubled the rate of N_2 fixation associated with forest litter. Improvements in straw management offer another means of increasing straw decomposition and associated N_2 fixation. As mentioned earlier, field experiments by Roper et al. (1989) indicated that smashing and lightly mixing the straw with surface soil resulted in the highest rates of N_2 fixation in the systems studied. Further improvements may be achieved by minimising soil disturbance so as to preserve anaerobic and microaerobic microsites for nitrogenase activity by N_2-fixing bacteria whilst at the same

time ensuring sufficient soil-straw contact to promote straw decomposition by other soil microorganisms.

Conclusions

Nitrogen fixation by bacteria using crop residues for energy may be a significant source of N in any cropping system where substantial amounts of plant material are left after harvest. Crop residues can be left on the soil, or they can be gathered up, transformed by composting and then spread back onto the soil. In either case naturally occurring, heterotrophic and phototrophic bacteria utilise the straw either directly, by the use of hemicelluloses and simple carbohydrates, or indirectly, following the decomposition of cellulose by decomposer microorganisms. Quantitative data on the contribution of phototrophic and heterotrophic bacteria to the N status of soils are limited and new technologies are needed, particularly in the field, in order to obtain accurate measurements of N_2 fixation (Gibson et al., 1988).

In the laboratory, N gains have been observed as a result of heterotrophic N_2 fixation associated with straw, but the quantities vary. Inoculation of straw with N_2-fixing and cellulolytic organisms, in small-scale systems, has produced substantial improvements in the efficiency of straw-associated N_2 fixation and therefore inoculation has the potential to significantly improve N gains in compost systems. Future research should focus on the selection of suitable cellulolytic and diazotrophic microorganisms and the development of practical methods for the efficient production of straw-biofertiliser. Further gains may be possible if cellulolytic and diazotrophic activity are combined in the same microorganism by introducing into diazotrophic bacteria, genes which encode for polysaccharase functions such as amylase, cellulase, pectinase, and xylanase (Richardson et al., 1991).

Inoculation is only likely to be successful in straw, such as in composts, where microbial populations are not established. In soils, inoculant microorganisms are less likely to survive and be active because of strong competition with the established, indigenous microflora, and therefore alternative strategies are needed to promote the decomposing and N_2-fixing activities of naturally-occurring microbial populations. The use of management protocols, such as liming to neutralise pH and promote microbial activity, straw treatment to ensure good microorganism-straw contact, and altered tillage practices to preserve soil structure and maintain oxygen gradients that support microbial activity, have all resulted in some improvements. Further research into new management systems may result in greater efficiencies.

Increasing N supply through biological N_2 fixation, should reduce the need for inorganic N fertilisers. Therefore, in the long term, provided appropriate strategies can be developed to overcome the negative aspects of straw retention (e.g. the development of appropriate crop rotations to break disease cycles), straw-associated N_2 fixation could provide significant financial savings to producers as well as contribute to the maintenance of soil health.

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